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Highly Sensitive and Selective Glucose Sensor Realized by Conducting Polymer Modified Nanoporous PtZn Alloy Electrode

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ABSTRACT :

Platinum is a well known element which shows a significant electrocatalytic activity in many important applications. In glucose sensor, because of the poisoning effect of reaction intermediates and the low surface area, the electrocatalytic activity towards the glucose oxidation is low which cause the low sensitivity. So, we fabricate a nanoporous PtZn alloy electrode by deposition-dissolution method. It provides a high active surface and a large enzyme encapsulating space per unit area when it used for an enzymatic glucose sensor. Glucose oxidase was immobilized on the electrode surface by capping with PEDOT composite and PPDA. The composite and PPDA also can exclude the interference ion such as ascorbic acid and uric acid to improve the selectivity. The surface area was determined by cyclic voltametry method and the surface structure and the element were analyzed by Scanning Electron Microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDX), respectively. The sensitivity is 13.5 µA/mM cm^2. It is a remarkable value with such simply prepared senor has high selectivity.

Keywords: Glucose biosensor, Conducting polymer, Amperometric detection, PEDOT, Electrochemical polymerization, Alloy

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1. Introduction

Electrochemical biosensor has received great attention so far. It is a very powerful analysis technique and has a wide range of applications, such as environmental monitoring, clinical purpose, biological and chemical analysis *etc*.[1-5] Because the diabetes mellitus is a very universal disease in our life, glucose biosensor is one of the most widely used apparatus. There are many kind of glucose biosensors published so far.[6-9] Among these, enzymatic glucose biosensor is one of the best decision due to rapid response, high sensitivity and selectivity.[10] Sensitivity is depended on the active surface area per unit area. Nanoporous materials were used for biosensors and other

applications such as fuel cell,[11] chemical sensor,[12] catalysis[13] for enhancing the surface area. Platinum based nanoporous materials with high surface area were very widely used because of the significant electrocatalytic activity. Several literatures were published that nanoporous Pt and Pt based alloy materials can prepare by alloying-dealloying method.[14-18] First, reduce the metal ions by electrochemical method to produce the metal deposition and alloying. Then, selectively oxidize one or two metal ions to achieve the metal ion dissolution. After these processes, the positions of the dissolved metals were emptied and led the surface area enhancement.

In this study, we prepared the nanoporous PtZn alloy electrode by deposition-dissolution method. First, Zn was electrochemically deposited on the Pt disk electrode to form PtZn alloy. Then, Zn was electrochemically dissolved from the electrode which

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didn't form the alloy. Then, nanoporous PtZn surface was achieved. And this prepared electrode was used for enzymatic glucose biosensor preparing. The conducting polymer encapsulation provided a highly sensitive electrode with rapid determination ability and good selectivity.

2. Experimental

2.1. Reagents and apparatus

Platinum disk electrode (2mm diam.) was purchased from HCH. Poly(3,4-ethylenedioxythiophene)/Polystyrene Sulfonic Acid (PEDOT/PSS) dispersion was obtained from Bayer. Polyvinyl alcohol (PVA) was donated by OCI (Korea). D-(+)-glucose was obtained from Fluka. Glucose oxidase (147900U/g), 1,3-penylenediamine (PDA) and Zinc chloride were purchased from Sigma-Aldrich. The phosphate buffer solution (PBS) was prepared with Na_2HPO_4, NaH_2PO_4 and NaCl. All solutions were prepared by DI water (18.2 $M\Omega cm^{-1}$).

Electrochemical experiments were done in conventional three-electrode system. Ag/AgCl (sat'd KCl) and Platinum plate were used for reference and counter electrode, respectively. Electrochemical experiments were performed with the potentiostat/galvanostat (EG&G M263Z) and glucose detection was done with the BAS 100B potentiostat (Bioanalytical System Inc., USA). The morphology of nanoporous PtZn was observed by Scanning Electron Microscope (SEM) and analyzed with the Energy Dispersive X-ray spectrometry (EDX).

2.2. Preparation of nanoporous PtZn electrode

Nanoporous PtZn alloy electrode was prepared by electrochemical deposition and dissolution method with platinum disk electrode (2 mm diam.). It was polished with 0.3 and 0.05 μm alumina slurry step by step. Then, washed with 30 vol% HNO_3 and DI water under sonication condition. Zn deposition condition was done by applying constant potential of −1.07 V vs. Ag/AgCl for 500 s at 60°C. The electrolyte was containing 0.4 M $ZnCl_2$ and 2.95 M KCl and the pH was 4.2. After Zn deposition, the electrode was washed with DI water and left in the desiccator for one day for alloying. Then, the electrode was put into 0.5M H_2SO_4 and HNO_3 solution to dissolve Zn which didn't form the PtZn alloy. The dissolution was done by applying constant potential of −0.1 V until the

current dropped to zero at room temperature.[15-17]

2.3. Preparation of glucose biosensor

The prepared nanoporous PtZn disk electrode was cleaned with DI water and dried in the air. Then 1 μL of 3000 unit/mL glucose oxidase (GOx) was dropped onto the nanoporous PtZn disk electrode surface and dried at 4°C. After drying, the electrode was capped with PEDOT composite and PPDA for enzyme immobilization and interference exclusion.[19] The PEDOT composite capping was progressed by dropping method. After drying, the electrode was put into the solution of 0.01 M 1,3-phenylenediamine/ 0.1 M $LiClO_4$/ acetonitrile for polymerization by cyclic voltammery method to form PPDA layer. The potential range was 0.3~1.1 V and the scan rate was 50 mV/s. Polymerization was progressed 3 cycles.[20] This prepared sensor was used for glucose detection.

2.4. Glucose detection

Glucose detection was progressed by chrono-amperometric method. The whole cell was assembled as in Fig. 1. Reference electrode was Ag/AgCl (sat'd KCl) and Pt plate was the counter electrode. Constant potential of 0.7 V was applied during glucose detection and the electrolyte was pH 0.7 PBS solution. After the current response stabilized, 0.2 mM glucose was injected every minute and checked the current response. After investigating the sensitivity of the sensor, interference effects from interfering species were tested.[19,20]
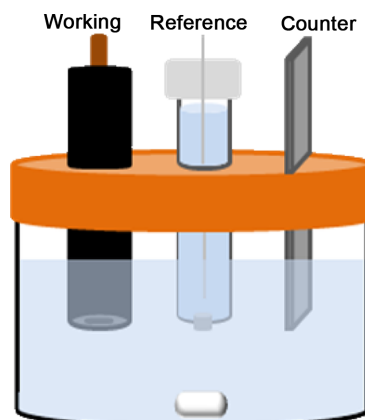

Fig. 1. This is the whole cell for electrochemical experiment. Reference and counter electrodes were Ag/AgCl (sat'd KCl) and Pt plate, respectively. The solution was stirred during glucose detection.

3. Results and Discussion

3.1. Morphology and formation of nanoporous PtZn

The morphology and element analysis of the electrode surface was verified by SEM and EDX. Fig. 2(a) is the image of bare Pt disk electrode. There are many irregular and shallow scratches. The scratches of bare Pt disk electrode may be due to the electrode polished with alumina slurry. Fig. 2(b) is the image of PtZn disk electrode. There are deeper irregular scratches and blocks appeared. The surface area looks enhanced after deposition-dissolution process. Fig. 2(c) is the EDX data of PtZn disk surface. EDX test shows that Zn was still remained. That means after Zn dissolution procedure, the Zn didn't dissolved perfectly. This result indicated that the Zn in PtZn form didn't dissolved at the oxidation potential in the electrolyte.

3.2. Cyclic voltametry (CV) characterization of nanoporous PtZn

In order to check the surface area enhancement of prepared PtZn alloy electrode, Cyclic voltametry method was employed. Fig. 3(a) is the cyclic

voltammogram of the nanoporous PtZn disk and bare Pt disk electrode in 0.5 M H_2SO_4. The potential range is 0.1 V to 1.5 V (vs. Ag/AgCl) and the scan rate is 50 mV/s. The insert is the magnified CV of bare Pt disk electrode. According to this figure, the current response obtained from PtZn alloy electrode was much higher than that from bare Pt electrode. It can demonstrate that the surface area of the PtZn alloy electrode was increased a lot compare to that of bare Pt electrode. The surface area enhancement can be numerically represented as Roughness Factor (RF). RF is the ratio between real surface area and geometrical area. It can calculated from hydrogen adsorption and desorption in Fig. 3(a). Substitute the conversion factor 210 $\mu C/cm^2$,[21-24] the RF of the nanoporous PtZn alloy electrode is 132.

Fig. 3(b) showed typical cyclic voltammograms for the methanol oxidation of PtZn disk and bare Pt disk electrode. The potential range is 0V to 1.0 V and the scan rate is 50 mV/s. The insert is the CV obtained by using bare Pt disk electrode. Because of the adsorption and reaction of the intermediate species, there are two oxidation peaks but no reduction peak appeared. In addition, poisoning species also attributed to only
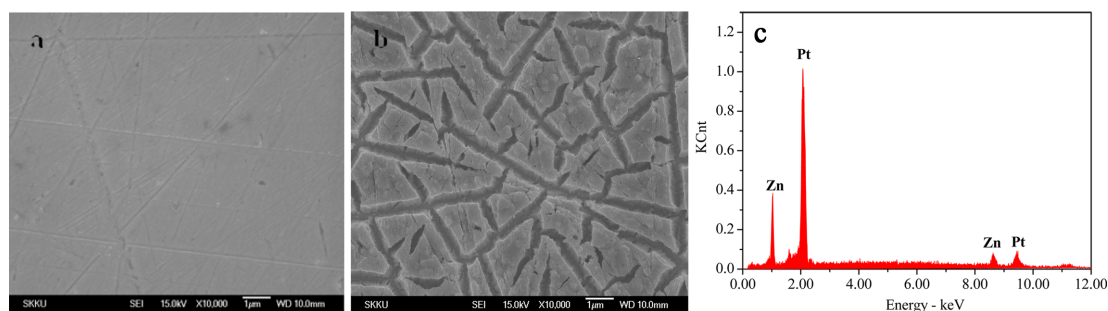

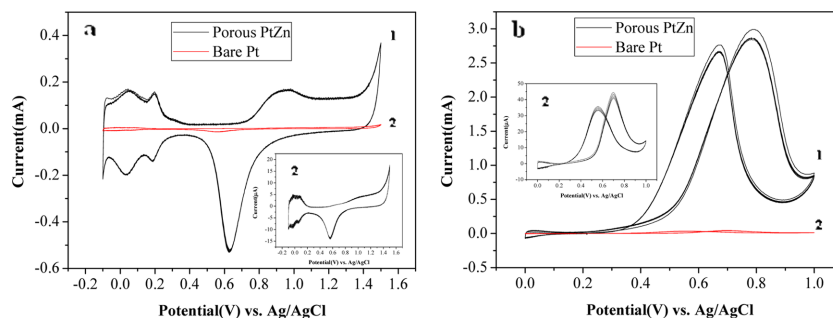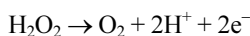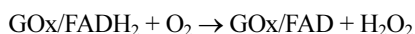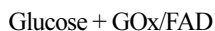**Fig. 2.** Surface morphology and formation element analyzed by SEM (a, b) and EDX (c).

Fig. 3. Cyclic voltammograms of nanoporous PtZn disk and bare Pt electrode in (a) 0.5 M H_2SO_4 solution; (b) 0.5 M methanol containing 0.2 M H_2SO_4. The insets are for bare electrodes and scan rate is 50 mV/s.

methanol oxidation reaction. According to this figure, the current response of PtZn alloy electrode is also much higher than the bare Pt disk electrode which can demonstrate that the surface area enhanced much.

3.3. Glucose detection

Glucose detection was performed with nanoporous PtZn disk electrode under constant potential of 0.7 V in 0.1 M pH 7.4 PBS solution and the solution was stirred during detection process. The reaction mechanism is defined as below.

Glucose + GOx/FAD
$\rightarrow$ Gluconolactone + GOx/FADH$_2$

GOx/FADH$_2$ + O$_2$ $\rightarrow$ GOx/FAD + H$_2$O$_2$

H$_2$O$_2$ $\rightarrow$ O$_2$ + 2H$^+$ + 2e$^-$

At the beginning, glucose is oxidized to gluconolactone by using Flavin Adenine Dinucleotide (FAD) as catalyst which has the GOx as active center. Then, the reduced form of GOx/FAD will react with the oxygen dissolved in the aqueous phase to product hydrogen peroxide. The response of glucose is identified by estimating the anodic current of the oxidation of hydrogen peroxide on the electrode.

Fig. 4(a) is the amperogram from glucose detection reaction. After stabilized under 0.7 V in 10 mL pH 7.4 PBS solution, 10 µL of 0.2 M glucose solution was injected every minute. It means the concentration of glucose in the PBS solution was increased 0.2 mM every minute. The response was appeared rapidly and linearly during the whole injection. Fig. 4(b) is the calibration curve obtained by using the data in Fig. 4(a). The sensitivity found to be 13.5 µA/mM cm^2 and compared to those from other works listed in Table 1. However, the sensitivity of our work was not the highest one, but one thing remarkable was the simplicity in preparation and still good for the glucose determination for medical purpose.

This biosensor was also tested in a solution containing several interfering species beside glucose. Ascorbic acid, uric acid, and acetaminophen were chosen as the interfering species. Because, the species are easily found in human blood. However, in human body, the concentration of glucose is thirty times higher than the interference at least.[25] There was almost no current increase existed when injecting interfering species which different from injecting glucose in Fig. 5. This has been known as screen effect of the conducting polymer film.[26]
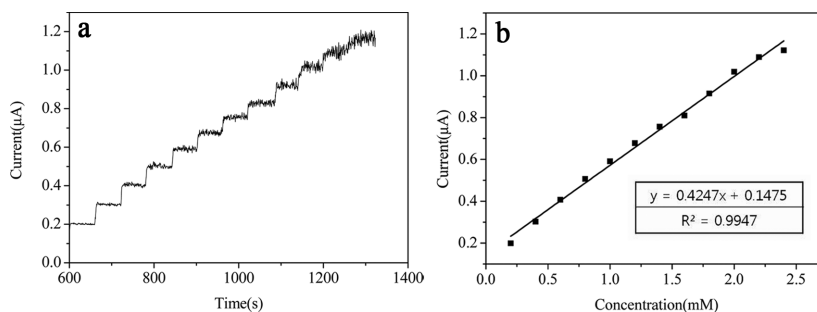

Fig. 4. (a) Amperometric response of prepared nanoporous PtZn disk based biosensor at an applied potential of 0.7 V vs. Ag/AgCl in the 0.1 M PBS solution. 0.2 mM glucose was injected every 1 min. (b) is the calibration curve.

Table 1. Sensitivity compared between this work and other works

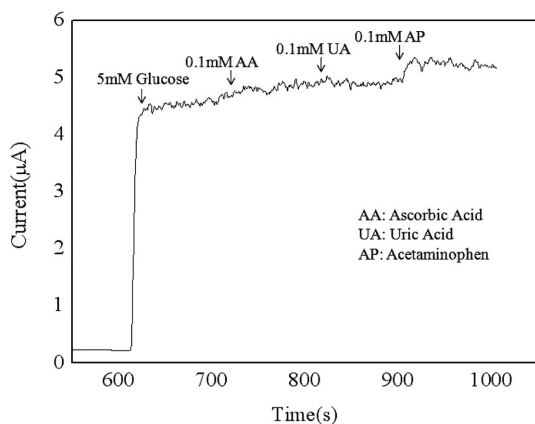| Fabrication method		Sensitivity
Porous PtZn electrode based biosensor modified by conducting polymer	This work	13.5 µA/mM cm^2
Covalent attachment of glucose oxidase to an Au electrode modified with gold nanoparticle for use as glucose biosensor	[27]	8.8 µA/mM cm^2
Multilayered construction of glucose oxidase and gold nanoparticles on Au electrode based on layer-by-layer covalent attachment	[28]	5.72 µA/mM cm^2
Enhanced sensitivity of a glucose sensor adopting polymer microtubule	[29]	9.33 µA/mM cm^2
In situ immobilization of glucose oxidase in chitosan-gold nanoparticle hybrid film on Prussian Blue modified electrode for high-sensitivity glucose detection	[30]	69.26 µA/mM cm^2

Fig. 5. (a) Amperometric response of prepared nanoporous PtZn disk based biosensor at an applied potential of 0.7 V vs. Ag/AgCl in the solution containing glucose and interference.

4. Conclusion

In this work, we successfully prepared nanoporous PtZn electrode by deposition-dissolution method and the surface enhanced by 134 times that compare with the bare electrode. However, Zn didn't dissolve perfectly. Then, we prepared the glucose biosensor with this electrode and progressed glucose detection process and interference test. The glucose detection sensitivity was 13.5 µA/mM cm^2 and the selectivity was well. According to my experiment, it can be demonstrated that we successfully prepared the glucose biosensor at rapid responding with high sensitivity and selectivity.

Acknowledgements

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