Preparation of glucose sensors using gold nanoparticles modified diamond electrode

Fachrurrazie, T A Ivandini and W Wibowo

Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia

Corresponding author's e-mail: ivandini.tri@sci.ui.ac.id

Abstract. A glucose sensor was successfully developed by immobilizing glucose oxidase (GOx) at boron-doped diamond (BDD) electrodes. Prior to GOx immobilization, the BDD was modified with gold nanoparticles (AuNPs). To immobilize AuNPs, the gold surface was modified to nitrogen termination. The characterization of the electrode surface was performed using an X-ray photoelectron spectroscopy and a scanning electron microscope, while the electrochemical properties of the enzyme electrode were characterized using cyclic voltammetry. Cyclic voltammograms of the prepared electrode for D-glucose in phosphate buffer solution pH 7 showed a new reduction peak at +0.16 V. The currents of the peak were linear in the concentration range of 0.1 M to 0.9 M, indicated that the GOx-AuNP-BDD can be applied for electrochemical glucose detection.

Keywords: Biosensors, glucose oxidase, gold nanoparticles, boron-doped diamond

1. Introduction

The basic for constructing a biosensor is to prepare the enzyme modified electrode. The enzyme needs to be immobilized on the electrode surface to obtain the electrochemical reaction immediately after enzyme bioactivity. However, by using enzyme, the accessibility of electron transferred between redox center and the surface of the electrode are limited by 3D protein structure of the enzyme.

On the other hand, boron-doped diamond (BDD) electrode offers special characteristics that required by electrochemical applications [1]. However, some challenges need to be resolved before it can be used as biosensors. To name a few, BDD has an inert surface, therefore modification on the surface needs to be performed.

In this research, glucose oxidase (GOx) was used as the biosensing/biocatalyst for the application in a glucose sensor. The redox active sites of this enzyme, i.e. flavin adenine dinucleotides (FADs) were located at the core center protein, thus limits its direct electron transfer to the electrode surface. It is known that Au offers good responses in electron transfer with enzyme hence increasing the surface area of Au in the system is important [2]. Accordingly, the surface of BDD was modified by gold nanoparticles (AuNPs) to achieve a more efficient electron transfer between the enzyme and BDD. Previously, our group has successfully developed AuNPs-modified BDD (AuNPs-BDD) for biochemical oxygen demand (BOD) sensing with yeasts as the biocatalysts [3-5]. Although the response of AuNPs-BDD electrode is smaller than that of bulk gold electrodes, the signal was generally clearer due to the smaller background current. In this work, the AuNPs-BDD was modified by GOx. This prepared electrode (GOx-AuNPs-BDD) was then investigated for a preliminary study of glucose sensors. The results showed good responses with linear peak currents to glucose concentrations.
2. Materials and methods

2.1. Materials
Glucose oxidase (GOx) from Aspergillus niger, D-glucose, H\textsubscript{AuCl}_4, NaBH\textsubscript{4}, sodium citrate, and NH\textsubscript{4}OH were supplied from Sigma or Sigma-Aldrich. Pt spiral was purchased from Nilaco Japan, while BDD was prepared in Keio University, Japan.

2.2. Methods
AuNPs was synthesized by mixing 10 mL of 0.5 mM H\textsubscript{AuCl}_4 with 5 mL of 0.1 M sodium citrate solution followed by a vigorous stirring at 25°C. After the addition of sodium citrate solution, the color of gold solution became transparent. After 1 h stirring, 0.5 mL fresh solution of NaBH\textsubscript{4} (0.1 M) was added dropwise, while stirring continuously for another hour. The appearance of pink color indicated the formation of AuNPs.

Prior to modify the BDD electrodes with AuNPs, the electrodes were immersed in the concentrated NH\textsubscript{4}OH solution and irradiated under a UV source (λ = 254 nm) for 6 h. Then, the electrodes were soaked in colloidal AuNPs for 20 min continued with drying at 60°C. XPS was used for the characterization process.

In order to immobilize GOx at AuNPs-BDD, 100 μL GOx solution (10 mg mL\textsuperscript{-1}) was poured to the surface of AuNPs-BDD and dried at 4°C using silica gel as water absorbent [6]. The modified electrodes were maintained at 4°C in dry condition when it is not in used. The electrode was then characterized by SEM.

The electrochemical analysis was performed in PBS pH 7 in the present of D-glucose to study the oxidation of glucose at the prepared electrodes.

3. Results and discussions
The color of H\textsubscript{AuCl}_4 solution changed from yellow to ruby red after the reduction reaction with NaBH\textsubscript{4} and sodium citrate, indicating the formation of AuNPs. Characterization of AuNPs by using UV-Vis spectroscopy shows a maximum absorption wavelength of AuNPs at 515 nm (figure 1a), while TEM image shows the main particle size around 30 nm (figure 1b). However, some particle sizes under 5 nm were also observed (figure 1). The nanoparticles size was not really homogenous, therefore, it was expected that the immobilization will cover the surface of BDD well, with the small size filled the space between the big size of nanoparticles.

![Figure 1](image1.png)
Figure 2. XPS Spectra of the GOx-AuNPs-BDD. The inset shows the magnification of gold binding energy at 107 eV and 109 eV.

Figure 3. (a) SEM Images of AuNPs-BDD and (b) GOx-AuNPs-BDD.

BDD surface is normally partly terminated by oxygen as the result of oxidation process in the air [3, 4]. In this case, the BDD surface needs to be modified to nitrogen termination by photochemistry reaction to exchange oxygen terminal with amine functional group (-NH₂) at the surface. Tian et al. [7] reported that self-assembly AuNPs-BDD can be formed at nitrogen-terminated BDD. It was proposed that covalent bonds between AuNPs and -NH₂ stabilize the position of AuNPs at the carbon surface [8] hence it was expected that a better stability of GOx at the surface of BDD can be generated.

XPS spectrum of GOx-modified AuNPs-BDD (figure 2) shows the diamond peak (C 1s) at binding energy of 288 eV and a peak corresponds to C-OH at 536 eV with an O:C ratio of 0.54. In addition, a couple of peaks at 107 eV and 109 eV were also observed (Inset of figure 2), indicating the existence of Au at the surface of BDD.

Furthermore, surface characterization of the GOx-modified BDD in comparison with BDD using SEM shows that the surface of BDD has been covered by a film, indicating that the GOx visible surface has been successfully immobilized at the surface of AuNP-BDD (figure 3).
Figure 4(a) shows cyclic voltammograms (CVs) in 0.1 M PBS pH 7 at BDD (dashed line) and AuNPs-BDD (solid line). Two reduction peaks at +1.10 V and +0.46 V were observed. These peaks are typical oxidation-reduction peaks of gold, indicating that AuNP was successfully deposited at BDD surface. These peaks were disappeared after immobilizing GOx at AuNPs-BDD surface (Figure 4b).

In the presence of glucose, CVs in figure 5 (color lines) show a reduction peak at +0.16 V at GOx-AuNP-BDD, which is typical reduction peak of H$_2$O$_2$, indicating that the GOx could be successfully deposited at AuNP-BDD electrodes. It is well known that the presence of GOx oxidizes glucose to be H$_2$O$_2$, which is easier to be reduced by gold electrodes. Various concentrations of glucose from 0.1 to 0.9 M were tested and the corresponding CVs are shown in Figure 5 (b).

**Figure 4.** Cyclic voltammograms of 0.1 M PBS pH 7 at BDD (dashed line) and AuNPs-BDD (solid line) in comparison with (b) that at GOx-AuNPs-BDD. The scan rate was 100 mV/s.

**Figure 5.** Cyclic voltammograms of 0.1 M PBS pH 7 in the absence (dashed line) and in presence of various concentrations of D-Glucose (0.1 M to 0.9 M, colored lines) at GOx-AuNPs-BDD. Figure (b) shows the magnification of the figure (a). The scan rate was 100 mV/s.
0.9 mM decreased this peak as shown in the right figure. The currents of this peak were linear with R2 of 0.99, indicating that the electrode can be applied as glucose sensors.

4. Conclusions
Glucose oxidase-modified AuNPs-BDD electrodes have been successfully prepared using self-assembly deposition of AuNPs with the size about 3 to 28 nm at N-terminated BDD. Furthermore, the prepared electrode can generate a reduction peak at +0.16 V in 0.5 M glucose solution, indicating that the electrode can be applied as glucose sensors.

References
[1] Ivandini T A and Einaga Y 2017 *Chem. Commun.* **53** 1338-47
[2] Holland J T, Lau C, Brozik S, Atanassov P and Banta S 2011 *J. Am. Chem. Soc.* **133** 19262-5
[3] Ivandini T A, Saepudin E, Wardah H, Harmesa Dewangga N and Einaga Y 2012 *Anal. Chem.* **84** 9825-32
[4] Ivandini T A, Wijaya L, Gunlazuardi J and Einaga Y 2012 *Makara J. Sci.* **16** 9-14
[5] Ivandini T A, Wicaksono W P, Saepudin E, Rismetov B and Einaga Y 2015 *Talanta* **134** 136-43
[6] Zhang Y, Li Y, Wu W, Jiang Y and Hu B 2014 *Biosensors Bioelectron.* **60** 271-6
[7] Tian R H, Rao T N, Einaga Y and Zhi J F 2006 *Chem. Mater.* **18** 939-45
[8] Folkers J P, Laibinis P E and Whitesides G M 1992 *Langmuir* **8** 1330-41