Preparation of hemoglobin-modified boron-doped diamond for acrylamide biosensors

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Abstract. Boron-doped diamond (BDD) electrode was modified with haemoglobin to develop electrochemical biosensors of acrylamide. Prior to modify with haemoglobin, the BDD was modified by gold nanoparticles to increase the affinity of BDD against haemoglobin. The electrochemical behaviour of the electrode in the presence of acrylamide was studied in comparison to haemoglobin-modified gold electrodes. Cyclic voltammetry indicated the optimum responses in 0.1 M sodium acetate buffer at pH 5. The responses were linear to the acrylamide concentration range of 5-50 μM with an estimated detection limit of 5.14 μM, suggesting that the electrode was promising for acrylamide biosensors.

Keywords: Acrylamide, Biosensor, Haemoglobin, Boron-Doped Diamond, Gold Nanoparticle

1. Introduction

In 2002, Swedish National Food Administration declared that heating process of foods which contains high carbohydrates such as potato chips, coffee, and bread leads to an acrylamide formation [1, 2]. The process is derived from the reaction between asparagine and reducing sugars, for example glucose and fructose, which is known as Maillard reaction [3-5]. Research over the years regarding the carcinogenic properties of acrylamide establishing the fact that acrylamide causes cancer in human [6, 7]. Therefore, International Agency For Research On Cancer [8] classifies acrylamide into a carcinogenic of Group 2A, while the European Commission [9] and the European Chemical Agency [10] classifies acrylamide as category 2 for carcinogenic and mutagenic.

The harmful effect of acrylamide leads to the requirement of a simple and accurate method for acrylamide detection. The conventional methods to detect acrylamide were generally based on chromatography techniques, including LC/MS, LC-MS/MS and GC/MS [2, 5, 11] which are considered as less efficient process. On the other hand, biosensors have been developed for a quick detection method of acrylamide [12].

Electrochemical biosensor of acrylamide was initially developed in 1997 [13]. Heavy metal ions or hemoglobin (Hb) was generally employed for the sensor elements. Comparison of these two methods showed that the performance of Hb-modified electrode was satisfactory. It was reported that the interaction formed at the double bond of acrylamide which are covalently bonded to the α-NH₂ at the N-terminal valine groups of Hb can be used as the responses) [2].

On the other hand, in comparison to other conventional electrodes, BDD is known to have some superior properties, such as wide potential window, low background currents, and high physical and chemical stability as well as good biocompatibility [14, 15]. In this study, Hb-modified BDD was
prepared for the applications as acrylamide biosensors. Initially, BDD was modified with gold nanoparticles in order to increase the affinity of Hb on the surface of BDD. Then, gold nanoparticles-modified BDD (AuNPs-BDD) was modified with Hb to form Hb-BDD. The results showed that Hb-BDD is promising to be applied for a sensor of acrylamide.

2. Materials and methods
Allylamine, human hemoglobin, CH$_3$COOH, CH$_3$COONa, HauCl$_4$, H$_2$SO$_4$, K$_2$HPO$_4$, KH$_2$PO$_4$, 1-propanol, and other chemicals were of analytical grade and supplied by Merck.

Prior to use, the BDD film was pre-treated with ultrasonication in 1-propanol for 15 min and high purity water for 15 min. Then, BDD was oxidized at +3.0 V (vs. Ag/AgCl) in 0.1 M H$_2$SO$_4$. After cleaned and dried, gold particles were electrodeposited on BDD surface to form AuNPs-BDD. Amperometry technique was used by applying a potential of +0.5 V (vs. Ag/AgCl) for 100 s in solution of 0.2 M H$_2$SO$_4$ containing of 2 mM HAuCl$_4$. Then, Hb-BDD was prepared by immersing the prepared AuNPs-BDD in a solution of 0.1 acetate buffer solution (ABS) pH 5 containing of 1.5 mg/mL human hemoglobin.

The electrochemical cell was filled with 4 mL of 0.1 M ABS containing acrylamide at pH 5. Then, a linear calibration curve for acrylamide was constructed from various acrylamide concentrations (5-50 μM). Cyclic voltammetry technique was performed with a potential range of -200 mV - 800 mV.

3. Results and discussion
3.1. Characterization of Hb-BDD electrodes
Figure 1a shows the XPS spectra of Hb-AuNPs BDD. Two peaks observed at 283 eV and 534 eV were identified as the C-C and C-O bonds of the BDD electrode, while the peak at 400 eV was attributed to the property of the C-N bonds from haemoglobin. In addition, a small peak at 90 eV was observed, indicating the presence of gold at the electrode surface. SEM characterization in figure 1b shows the presence of fiber of haemoglobin that was immobilized on the electrode surface, indicating the Hb-AuNP BDD can be successfully prepared.

AuNPs deposited on the electrodes BDD is assured to increase the direct electron transfer between hemoglobin and bulk electrodes [16]. On the other hand, the presence of AuNP was expected to facilitate the adsorption of hemoglobin onto AuNPs through the interactions between cysteine and NH$_2$-lysine residues of hemoglobin [17].

![Figure 1. (a) XPS spectrum and (b) SEM image of Hb-AuNPs BDD.](image-url)
In order to study the electrochemical properties of the Hb-modified gold electrode, cyclic voltammetry was investigated at various pHs. While an oxidation peak at +0.45 V was observed at pH 5, no peak can be observed at pH 7 and pH 9 (figure 2). Therefore, the next experiments were conducted at pH 5. Conversion to the current density and power density is shown in figure 2.

3.2. The response of Hb-AuNPs-BDD electrode in the presence of acrylamide
Cyclic voltammetry of Hb-AuNPs-BDD electrode was studied in 0.1 M ABS pH 5 at a scan rate of 100 mV/s (figure 3a). Hemoglobin oxidation peak was observed at a potential of 0.43V (vs. Ag/AgCl). The oxidation peak decreased in the presence of acrylamide. Furthermore, investigation using various concentrations of acrylamide (0 – 50 μM) indicated that the electrode could be used for acrylamide sensors. A linear calibration curve ($r^2 = 0.97$) with an LOD of 8.5 μM can be achieved (figure 3b), indicating that hemoglobin-modified BDD is promising to be developed for the detection method of acrylamide.

4. Conclusions
BDD electrode was successfully modified with hemoglobin after the BDD modification with gold nanoparticles (Hb-AuNP-BDD). A linear calibration curve can be obtained at pH 5 at various acrylamide concentrations (0-50 μM) with an estimated LOD of 8.5 μM using this electrode.
Acknowledgements
This work was funded by Hibah Kompetensi Publikasi Internasional Terindeks Universitas Indonesia 2016, Contract No. 2016/UN2.R12/HKP.05.00/2016.

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