Modification of boron-doped diamond with gold through wet-chemical seeding and electrodeposition techniques for the application of acrylamide biosensor

L E Anggraini, E Saepudin and T A Ivandini

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

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

Abstract. Acrylamide is known as carcinogenic and neurotoxin substrates. An alternative method for acrylamide detection is by using hemoglobin-based biosensors as it can provide a simple, rapid, and sensitive method. In this research, the electrodes for biosensor of acrylamide were prepared by modifying boron-doped diamond (BDD) with gold and hemoglobin (Hb). Wet-chemical seeding followed by electrochemical overgrowth of seeds technique was performed to modify the BDD electrodes with gold. The characterization with Scanning Electron Microscopy-Energy Dispersive Spectroscopy X-Ray (SEM-EDS) showed the gold coverage of 12.74% on the BDD surface. The gold-modified BDD was then modified with 0.25 mM Hb. The prepared sensor can be successfully applied in the concentration range of acrylamide from 0.6 to 6 μM with high linearity (R = 0.9901) and an estimated limit of detection of 0.845 μM.

Keywords: acrylamide, biosensor, boron-doped diamond, gold, hemoglobin

1. Introduction

Carbohydrate-rich foods, such as potatoes, bread, coffee, cigarettes, and cereal products, can stimulate the formation of carcinogenic compounds called acrylamide through the frying or roasting process at high temperatures [1–3]. The acrylamide formation occurs through Maillard reaction, involving the heating of asparagines and reducing sugars [1,3–7]. Research over the years proves that acrylamide is a neurotoxin and toxic compound and also rapidly absorbed by humans and distributed in brain, thymus, heart and liver [1,2,4,5,8]. Accordingly, this substance is categorized as “probably carcinogenic to humans” by the International Agency on Research on Cancer (IARC) [2].

Various analytical methods, such as chromatography and mass spectroscopy, are available for acrylamide detection. But these methods are not applicable for common people. On the contrary, biosensor methods can overcome these problems due to their simple, rapid, sensitive and specific applications [8,9–13].

In this work, hemoglobin (Hb) is used as the biosensing since Hb and acrylamide interacts each other through N-terminal valine of Hb. This interaction decreases the electrochemical signal of acrylamide. On the other hand, boron-doped diamond (BDD) is widely applied in electrochemical applications because it has stable surface morphology and microstructures, low background currents, and wide potential windows [9,14–16]. However, BDD has low sensitivity to hemoglobin, therefore, modification with gold is required to facilitate the electron transfer between Hb and BDD [4,16–18].

Furthermore, a common method to modify gold onto BDD surface, i.e. electrodeposition, often produces poor electrode stability [9,18,19]. Therefore, in this work, the method to prepare gold-modified BDD (Au-BDD) electrode was performed through wet-chemical seeding and electrochemical overgrowth of seeds techniques to increase the electrode’s stability.
2. Materials and methods

2.1. Reagents and instruments
The polycrystalline BDD films (B:C ratio of the precursor gas was 1:1000) were supplied from Einaga’s Laboratory, Keio University, Japan. The films were prepared in chemical vapor deposition method by using methane as the carbon source and trimethoxy borane as the boron source. Detail of the preparation was described here [20]. Human hemoglobin H7379 and acrylamide were obtained from Sigma-Aldrich, while sodium borohydride, sodium hydroxide, isopropanol, n-hexane, chloroauric acid tetrahydrate (HAuCl₄·H₂O) and other chemicals were purchased from Merck. Acetate buffer solution (ABS) pH 5 was made by mixing acetic acid and sodium acetate. Ultra-pure deionized water was obtained from Millipore Direct® Q5-UV at resistivity of 18.2 MΩ·cm.

Potentiostat (Metrohm DropSens) with three electrodes system was used, served as working electrode was Hb/Au-BDD, while counter and reference electrodes were Pt wire and Ag/AgCl, respectively. Rapid Thermal Annealing (RTA), Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS) analysis (JEOL JCM-6000PLUS NeoScope), Raman Spectrophotometer (Olympus), and High Performance Liquid Chromatography (LC210) were utilized in this research.

2.2. Modification of BDD with gold by using wet-chemical seeding and electrochemical overgrowth of seeds techniques
To modify the BDD, solutions of 1M NaBH₄ in 0.1M NaOH and 1mM HAuCl₄ were subsequently dropped onto the BDD surface. After 24 h, the BDD was washed and dried. The deposition procedure was repeated 3 times. Then, the amperometric deposition was conducted using 1 nM HAuCl₄ solution at a constant potential of -0.2 V. Next, rapid thermal annealing at 700 °C in N₂ atmosphere for 5 min was conducted. After annealing, the electrode was refreshed by using cyclic voltammetry (CV) in 0.1M HSO₄ for 50 cycles to activate the electrode, followed by amperometric deposition at -0.2 V. [8]. Stability of Au-BDD electrode was examined with 30 min sonicated and 100 times CV, while characterization of the electrode was performed by using CV and SEM-EDS.

2.3. Preparation of hemoglobin modified Au-BDD (Hb/Au-BDD) electrode
Hb powder was dissolved in 0.1 M acetate buffer solution (ABS) pH 5. Volume of 15 µL Hb solution was dropped onto Au-BDD electrode, then stored at 4 °C for 24 h to keep the Hb condition from being damaged and remain stable to the surface of the electrode. Characterization was performed by SEM EDS.

2.4. Response measurements of Hb/Au-BDD electrode
The measurements of acrylamide biosensor were performed by using CV technique in the potential range of -0.2 to +0.8 V, served as working electrode was Hb/Au-BDD, while counter and reference electrodes were Pt wire and Ag/AgCl, respectively. The scan rate was 0.1 V/s. Acrylamide standard solutions were prepared by dissolution of acrylamide powder in ABS pH 5.

3. Results and discussion

3.1. Preparation of Au-BDD electrode by using wet-chemical seeding and electrochemical overgrowth of seeds technique
Theoretically, there will be a reaction between NaBH₄ and HAuCl₄ on the surface of the BDD electrodes according to the following reaction:

\[
\text{HAuCl}_4 + 4 \text{NaBH}_4 + 12 \text{H}_2\text{O} \rightarrow \text{Au} + 4 \text{B(OH)}_3 + 4 \text{NaCl} + 29 \text{H}_2
\]

This process deposited gold particles on the surface of the BDD by physical adsorption. Then, the electrodeposition was carried out at a potential range of -0.5 to +1.5 V, where a reduction reaction from Au⁺ to Au occurred to form gold metal particles on the surface of BDD, particularly at the gold particles. Figure 1 shows the comparison of the cyclic voltammogram of BDD before modification (red line), after wet chemical seeding (green line), and after electrodeposition (black line). The calcination by using rapid thermal annealing (RTA) at 700 °C for 5 min in N₂ atmosphere was then conducted to improve the growth of gold particles on the BDD surface. N₂ atmosphere was used to prevent the formation of metal oxides when heated at this high temperature. However, because of this treatment, the electrode surface was inactive as shown by lower gold oxidation-reduction peak currents after annealing (light blue line).
Figure 1. Cyclic voltammograms performed in 0.1 M acetate buffer solution pH 5 at scan rate 100 mV/s of at BDD before modification (red line), after wet chemical seeding of gold (green line), after first electrodeposition black line), after annealing (light blue), after refreshing treatment (orange line), and after activation by using 2\textsuperscript{nd} electrodeposition (blue line).

Figure 2. SEM images every steps Au-BDD electrodes.

CV was performed to refresh the Au-BDD surface by repeating the oxidation-reduction in a 0.1M H\textsubscript{2}SO\textsubscript{4} solution for 50 times. After the treatment, recovery of the peak was observed as shown in figure 1 (orange line), indicating the electrode was electrochemically active. However, the electrochemical redeposition of gold using HAuCl\textsubscript{4} solution was necessary to recover the current response of gold (blue line).

SEM images were conducted to study the change on the BDD surface after modification. Figure 2 shows the SEM images of the BDD surface before and at every step after modification. The figure shows that at the seeding step, less gold particles were deposited, while the number and size of gold particles increased after the first electrodeposition. Next, the annealing treatment homogenized the particle size and distribution, which was then randomly spread after refreshing treatment. However, after the 2\textsuperscript{nd} electrodeposition the particles were more homogeneously deposited.

Sonication of the Au-BDD was performed for 30 min to examine the stability of the deposited gold. The CV performed in ABS pH 5 showed no change of the peak in the voltammograms before and after sonication (figure 3a). The current decrease was around 1.95 %. Furthermore, CV was also applied for repetitive 100 cycles in a solution of ABS pH 5, where a slightly decrease of the peak current was observed with an RSD of 1.35 % (figure 3b). The results indicated a good stability of the Au-BDD.
3.2. Preparation of hemoglobin modified Au-BDD (Hb/Au-BDD) electrode

After obtaining Au-BDD electrode with homogenous gold distribution and high stability, Hb was modified at the Au-BDD. The interaction between Hb and Au-BDD electrode occurs between the amino acid cysteine or NH₄⁺-lysine residues of Hb structure and gold surface through the amino and thiol groups [3,4,18]. In the presence of acrylamide, the interaction was disturbed, resulting in the decrease of Hb currents due to the stronger attraction of amine groups in Hb toward acrylamide. This interaction causes a stunted redox system that occurs in Fe atoms in hemoglobin, so that the oxidation current response and the reduction of Fe(III)/(II) decreases.

Figure 4a shows the voltammograms of 0.2M ABS pH 5 in the presence of various concentrations (0.6–6.0μM) of acrylamide. Couple of oxidation reduction peaks were observed at the potentials of +0.5 and +0.3 V, respectively, reflecting the oxidation-reduction of Fe(III)/Fe(II) in Hb structure. Furthermore, the decrease of the oxidation currents peak was linear (R² = 0.991) with the equation of y = -0.035x + 5.6115 (figure 4b) suggested that the sensors can be used for detection of acrylamide. The estimated detection limit of 0.845 μM was achieved. The repetitive measurements on 1μM acrylamide show an RSD of 1.073 %. The 5 times measurements, suggested the excellent stability of the electrode.
Table 1. Comparison of various electrochemical biosensors for acrylamide detection

| Electrode                                      | Optimum pH | Linear Range (M) | LOD (M)     | R       | Sensitivity (μA/μM.cm²) | RSD repeatability (%) | Reference          |
|------------------------------------------------|------------|------------------|-------------|---------|------------------------|-----------------------|---------------------|
| Hemoglobin/Au modified BDD (seeding method)   | 5          | 6 x 10⁻⁶ to 6 x 10⁻⁷ | 8.45 x 10⁻⁹ | 0.99    | 0.0350                 | 1.073                 | This paper          |
| Hemoglobin/Au modified BDD (electrodeposition method) | 5          | 5 x 10⁻⁶ to 5 x 10⁻⁷ | 5.14 x 10⁻⁹ | 0.97    | 0.0016                 | -                     | [9]                 |
| HbNPs modified Au Polycrystalline             | 5          | 1 x 10⁻¹ to 1 x 10⁻¹ | 1 x 10⁻⁵    | 0.99    | -                      | 3.85                  | [8]                 |
| Polyethersulphone double membrane             | 7.2        | 1 x 10⁻¹ to 1 x 10⁻¹ | 6.31 x 10⁻⁶ | 0.99    | -                      | -                     | [10]                |
| Polycarbonate double membrane                 | 7.2        | 5 x 10⁻⁴ to 5 x 10⁻⁴ | 9.12 x 10⁻⁷ | 0.99    | -                      | -                     | [10]                |
| Multi-walled carbon nanotube/CoNPs/Polyaniline | 5.5        | 75 x 10⁻⁸ to 2 x 10⁻¹ | 2 x 10⁻⁸    | 0.99    | 2.35                   |                       | [11]                |
| Screen printed carboxyl single-walled carbon nanotubes | -          | 1 x 10⁻¹ to 2 x 10⁻¹ | 3 x 10⁻⁸    | 0.99    | 0.009                  | -                     | [12]                |
| Multi-walled carbon nanotube/FeONPs/chitosan composite | 5          | 9 x 10⁻¹ to 2 x 10⁻¹ | 2 x 10⁻⁹    | 0.99    | 0.0024                 | 2.2                   | [13]                |

Table 1 shows comparison of various biosensor electrodes for detecting acrylamide compound. The results show that current electrode (hemoglobin/gold modified BDD through seeding method) has a better sensitivity (0.035 μA/μM.cm²) and stability (1.073 %) compared to other electrodes. Although the detection limit of the current sensor was a bit higher than previous biosensor based on Au Polycrystalline modified HbNPs [8], multi-walled carbon nanotube/CoNPs/Polyaniline [10], screen printed carboxyl single-walled carbon nanotubes [12], and multi-walled carbon nanotubes/FeONPs/chitosan composite [13], but slightly lower than previous biosensor based on Hb/Au modified BDD [9], polyethersulphone double membrane [10], and polycarbonate double membrane [10].

4. Conclusions
Gold-modified boron-doped diamond (Au-BDD) was prepared by wet-chemical seeding and electrodeposition technique in order to produce excellent particles stability and homogeneity. Stability was demonstrated by the repeated sonication and voltammetry cyclic, while the homogeneity of the particles was confirmed by SEM characterization. Hemoglobin-modified on the surface of the Au-BDD electrode was then applied for acrylamide sensors by using cyclic voltammetry. The results showed that Hb-modified Au-BDD electrode can be applied as acrylamide biosensor and able to detect acrylamide with linear correlation of 0.901 in the concentration range of 0.6 to 6.0μM.

Acknowledgements
This work was funded by Hibah Kompetensi Publikasi Internasional Terindeks Universitas Indonesia, Contract No. 2273/UN2.R3.1/HKP.05.00/2018.
References
[1] Tareke E, Rydberg P, Karlsson P, Eriksson S and Törnqvist M 2002 J. Agric. Food. Chem. 50 4998–5006
[2] Lineback D R, Coughlin J R and Stadler R H 2012 Annu. Rev. Food Sci. Technol 3 15–35
[3] Stobiecka A, Radecka H and Radecki J 2007 Biosens. Bioelectron. 22 2165–70
[4] Hu Q, Xu X, Li Z, Zhang Y, Wang J, Fu Y and Li Y 2014 Biosens. Bioelectron. 54 64–71
[5] Stadler R H, Blank I, Varga N, Robert F, Hau J, Guy P A, Robert M C and Riediker S 2002 Nature 419 449–50
[6] Mottram D S, Wedzicha B L and Dodson A T 2002 Nature 419 448–9
[7] Zyzak D V et al. 2003 J. Agric. Food Chem. 51 4782–7
[8] Yadav N, Chilliar A K and Pundir C S 2018 Int. J. Biol. Macromol. 107 1000–13
[9] Umam K, Saepudin E and Ivandini T A 2017 IOP Conf. Ser.: Mater. Sci. Eng. 188 012006
[10] Silva N A F, Matos M J, Karmali A and Rocha M M 2011 Port. Electrochim. Acta 29 361–73
[11] Batra B, Lata S, Sharma M and Pundir C S 2013 Anal. Biochem. 433 210–7
[12] González-Fuentes F J, Maríquez J, Godínez L A, Escarpa A and Mendoza S 2014 Electroanalysis 26 1039–44
[13] Batra B, Lata S, Sharma M and Pundir C S 2013 Bioprocess. Biosyst. Eng. 36 1591–9
[14] Suzuki A, Ivandini T A, Kamiya A, Nomura S, Yamanuki M, Matsumoto K, Fujishima A and Einaga Y 2007 Sens. Actuators B: Chem. 120 500–7
[15] Gao F and Yang N 2013 Electrochim. Acta 112 493–9
[16] Li M, Zhao G, Geng R dan Hu H 2008 Bioelectrochem. 74 217–21
[17] Friedman M 2003 J. Agric. Food Chem. 51 4504–26
[18] Garabagiu S and Mihailescu G 2011 J. Electroanal. Chem. 659 196–200
[19] Wulandari R, Saepudin E and Ivandini T A 2019 Sens. Mater. 31 1105–17
[20] Ivandini T A and Einaga Y 2017 Heavy metal sensing based on diamond electrodes Carbon-Based Nanosensor Technology (Springer Series on Chemical Sensors: Methods and Applications) vol 17 ed. C Kranz (Cham: Springer)