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High stable Hb/Pt/BDD electrode for determining acrylamide in coffee samples

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Abstract. A novel electrochemical hemoglobin-platinum-modified boron-doped diamond (Hb/Pt/BDD) biosensor was developed for the detection of acrylamide (AA) in coffee samples. The Hb-Pt-BDD electrode was characterized by cyclic voltammetry (CV), scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS), X-ray diffraction (XRD), Raman spectroscopy, and X-ray photoelectrons (XPS). The cyclic voltammetry of Hb-Pt-BDD in 0.2 M sodium acetate buffer (ABS, pH 4.8) containing acrylamide in the concentration range of 0.00213 to 0.00711 ppb, showed linear responses with a detection limit of 0.00155 ppb. The excellent stability of the prepared Pt-modified BDD was proven through the Pt-BDD reusability by removing the Hb adduct without eliminating Pt on the BDD surface. Finally, employing the biosensor was proposed to determine AA in 1 g of coffee showed the AA content of 15.55 ppb. The result was comparable with the reference method using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Keywords: Acrylamide, biosensors, boron-doped diamond, platinum, hemoglobin, coffee samples

1. Introduction

Previous studies have discussed for the preparation of Hb/Pt/BDD electrodes as stable acrylamide sensors [1]. Acrylamide (AA) is generated from the reaction among reducing sugars namely, glucose and the amino acid, i.e. asparagine. The Maillard reaction mechanism was suggested to promote the AA formation in high starch foods during cooking at high temperatures [2-4]. It is also well-known that acrylamide compounds have been detected in coffee, French fries and some other common foods [5-7].

The high level of coffee consumption in many places in the world, making coffee as a source of daily exposure to acrylamide. Recently, the European Food Control Agency (EFSA) stated that roasted coffee and potato-based foods became one of the main sources of acrylamide exposure in the human body [8]. The acrylamide content in coffee is around 3–68 (µg/kg), while the Tolerable Daily Intake (TDI) or permitted daily AA intake is 40 µg/kg (pbb) per day for neurotoxicity and 2.6 µg/kg (ppb) per day to prevent cancer. Although, according to the Environmental Health Hazard Assessment (OEAHHA), one of the EPA divisions located in California, United States has determined that 0.2 µg/kg (ppb) days of acrylamide are not carcinogenic [8].On the other hand, several studies found that acrylamide has correlation to prostate cancer [9, 10], pancreatic cancer [11, 12], breast cancer [13-15], esophageal

cancer [16], ovarian cancer and brain cancer [9, 17]. Only few reports were recorded for the electrochemical analysis on the hemoglobin adducts with modified BDD electrodes [1], therefore, in this work we used Hb/Pt/BDD electrodes for detection of AA in coffee samples.

2. Experimental

2.1. Materials and instrument

The laboratory-made of BDD films was set up in Keio University, Japan by using microwave plasma-assisted chemical vapour deposition (MPCVD, CORNES Technologies/AZTeX-5400)with a 0.1 % boron-to-carbon ratio as the precursor solution [18, 19]. A (100) silicon wafer was used as the support. The BDD film thickness of around 5 μm was observed, $H_2PtCl_6 \cdot 6H_2O$, $NaBH_4$, and NaOH were supplied by Wako Inc. (Japan), while human Hb H7379 and acrylamide were obtained from Sigma-Aldrich. All chemicals were applied as received without further purification.

Electrochemical trials were performed with an EDAQ Potentiostat. A three-electrode system was employed with Ag/AgCl reference and Pt counter electrodes. The working electrode was a BDD modifed with platinum nanoparticles (PtNP) and Hb. SEM images were registered by a EVO MA 10 Carl Zeiss Microscopy Gmbh,Germany. LCMS/MS used were LC (Shimadzu), MS (AB SCIEX), pumps (LC-20AD), Autosampler (SIL-20A HT), column ovens (CTO-20AC), detectors (MS/MS AB SCIEX 3200 Q TRAP).

2.2. Modificationof PtNP and Hb at BDD electrode

The deposition of PtNP on BDD was performed by following the previous reports [20]. Briefly, 10 μL $NaBH_4$ solution was dropped onto the BDD electrode, followed by 40 μL 1 mM H_2PtCl_6 solution. After washed and dried, electrochemical overgrowth of Pt seeds was conducted in 5 mL 1.0 mM H_2PtCl_6 solution gradually for 15 min at a constant potential of -0.2 V (vs. Ag/AgCl). Following the overgrowth, the PtNP coated BDD electrode was handled with thermal annealing at 700 °C for 5 min in N_2 atmosphere. The sample was then electrochemically refreshed by using cyclic voltammetry at the potential between -0.5 V and 1.5 V at a scan rate of 200 mV/s for 100 cycles. A further overgrowth was performed using a deposition voltage of -0.2 V gradually for 15 min to renew the Pt/BDD surface. Then, 10 μL 0.2 M acetate buffer solution (ABS) pH 4.9 containing 0.15 mM Hb was dropped onto Pt/BDD electrodes for 24 h to obtain Hb/Pt/BDD modified electrode. The electrode was rinsed, dried, and kept in air at 4 °C when not used.

2.3. Detection of acrylamide in sample coffee using Hb/Pt/BDD electrode

The electrode was examined for the detection of acrylamide by using 1 g of Luwak Toraja coffee sample dissolved in 10 mL of boiling water. After separation, 1 mL of coffee filtrate was diluted to 50 mL using ABS solution, then, characterized using a Hb/Pt/BDD sensor. Cyclic voltammetry (CV) techniquue in a potential range between -0.5 V and 1.5 V at a scan rate of 100 mV/s was applied. Validation was performed by using LCMS/MS [21, 22]. The mobile phase used was acetonitrile 70 % (B) and 30 % water (A), while the column was PHENOMENEX AQUA 5u C18 125A (50 × 2 mm 5 micron). The flow rate of 0.4 mL min-1 and the constant column temperature at 40 °C were applied.

3. Results and discussion

3.1. BDD electrode modification with PtNP and Hb

The addition of $NaBH_4$ solution and H_2PtCl_6 solution was performed on the BDD surface to implant Pt over the BDD surface. The presence of $NaBH_4$ produces a tensile force between BH_4^- on $NaBH_4$ with H^+ on BDD surface, which will increase the adsorption of $NaBH_4$ on BDD surface. Electrochemical overgrowth of Pt seeds was then performed using electrodeposition to grow and uniform the Pt layer on

the BDD surface with a size and density that can be controlled so that Pt was homogeneously distributed on the BDD surface. The thermal annealing at 700 °C was then carried out for 5 min in N_2 atmosphere. N_2 gas was expected to prevent the formation of metal oxides. Then, electrochemically refreshed by using CV at the potential between -0.5 V and 1.5 V at a scan rate of 200 mV/s for 100 cycles in order to eliminate passive surface formation of Pt after high temperature treatment. A further overgrowth was performed using a deposition voltage of -0.2 V gradually for 15 min to renew the Pt/BDD surface. Then, 10 µL 0.2M ABS solution pH 4.9 containing 0.15 mM Hb was dropped onto Pt/BDD electrodes for 24 h to obtain Hb/Pt/BDD modified electrode. This BDD electrode modification with PtNP and Hb has been successfully carried out as evidenced by the results of SEM-EDS in figure 1 and table 1. SEM results in figure 1 shows that the Hb/Pt/BDD electrodes -before being contacted on acrylamide- was covered by Hb and Pt. EDS (table 1) shows the electrode characterization before being used for acrylamide detection. Table 1 confirmed that Pt and Fe could be deposited on the surface of BDD.

3.2. Acrylamide calibration curves with Hb/Pt/BDD electrodes

Hb/Pt/BDD electrodes were then tested for its performance on the standard acrylamide samples with various concentrations using the cyclic voltammetry. Measurements were made from a potential range of -0.5 to 1.5 V using 0.2 M ABS electrolytes containing acrylamide with concentration variation of 0; 0.00213; 0.00284; 0.00355; 0.00426; 0.00497; 0.00568; 0.00639 and 0.00711 ppb.

The results of the Hb/Pt/BDD electrode performance test on the standard acrylamide solutions in figure 2 show that there was a decrease in the peak of the oxidation current at the potential peak of +1.024, which attributed to Fe^{2+} oxidation. Increasing the concentrations of acrylamide decreases this oxidation peak. This is because the interaction between the N-terminal valine amino group of the Hb structure and the acrylamide compound affects the oxidation properties of Hb.

Acrylamide forms adducts with Hb as a result of the reaction between the -NH_2 group of valine N-terminal Hb. The formation of this adduct is related to the structure change of Hb. This structural change may be responsible for the decrease in the accessibility of the redox-active center Hb moving on

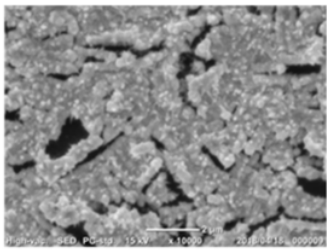
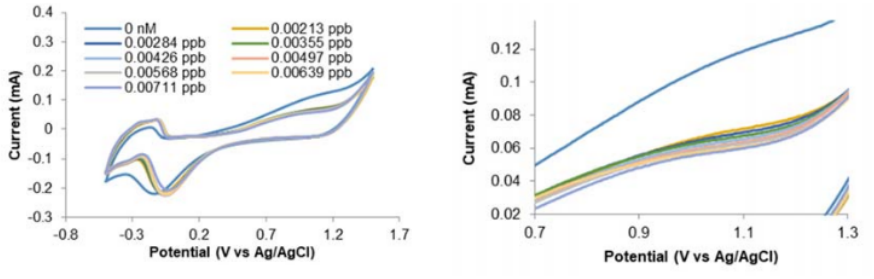


Figure 1. SEM image of Hb/Pt/BDD electrode.

Table 1. Mass composition of Hb/Pt/BDD in figure 1 Characterized by EDS.

Element	Mass %	Atomic number %
B	ND	ND
C	16.77	72.39
O	1.81	5.88
Fe	0.15	0.14
Pt	81.27	21.60



(a) (b)

Figure 2. (a) Hb/Pt/BDD electrode voltammogram with various concentrations of standard acrylamide in 0.2 M acetate buffer solution at a scan rate of 100 mV/s at Hb/Pt/BDD, (b) with the magnification.

the surface of the electrode, which causes a decrease in current. Adsorption of Hb-acrylamide changes the Hb electroactivity and the response that results from biosensors. Investigations of Pt/BDD electrodes modified with Hb displayed the quasi-reversible electrochemical reactions of Hb-Fe^{3+}/Hb-Fe^{2+}. The obtained results indicate that the decrease of the Fe^{2+} oxidation peak can be employed as analytical signal for acrylamide biosensors.

The results of the Hb/Pt/BDD electrode performance test on a standard acrylamide sample are shown in figure 3 in the form of a calibration curve. The results of the measurement of standard acrylamide compounds at various concentrations using Hb/Pt/BDD electrodes obtained R^2 = 0.996. Hb/Pt/BDD electrodes were used to measure standard acrylamide with 6 times repetition. An estimated LOD of 0.00155 ppb was obtained with a standard deviation of 0.00111.

3.3. Hb/Pt/BDD electrode performance test on Toraja Luwak coffee samples

Hb/Pt/BDD electrodes were then tested for their performance on coffee samples using the cyclic voltammetry. Measurements were made from a potential range of -0.5 to +1.5 V and scanrate was 100 mV/s in 0.2 M ABS pH 4.8. The sample was prepared with 1 g of Luwak Toraja coffee powder sample dissolved in 10 mL. Then, 1 mL of the sample was diluted up to 10,000 times in ABS. The solution was then measured using Hb/Pt/BDD electrodes. The voltammogram shown in figure 4 displays a peak oxidation potential of Fe^{2+} around +1.1 V. The measurement results of coffee samples using Hb/Pt/BDD electrodes are used to measure a sample with 3 repetitions. From the results of the Hb/Pt/BDD electrode performance, the calculations using a standard calibration curve acrylamide with CV show acrylamide levels in coffee samples of 15.55 ppb (oxidation current), after multiplied by the dilution factor.

3.4. Validation detection of acrylamide in coffee sample with LC-MS/MS

The measurement results of coffee samples using Hb/Pt/BDD electrodes were then validated by the LC-MS/MS. One g of Luwak Toraja coffee powder sample was dissolved in 10 mL water followed by boiling. The coffee solution was filtered using filter paper, 1 mL was taken, then diluted to 50 mL in a 0.1 % solution of formic acidsolution. The solution was then placed in a sample bottle. Series of standard acrylamide solutions were made with various concentrations (10, 50, 100, 150 and 200) ppb, then characterized using LC-MS/MS. LC-MS/MS was conditioned in the mobile phase of acetonitrile and aquabidest. The analysis time of 2 min was required with a peak flow rate of 0.4 mL/min, the peak was detected at 71.90. In figure 5, it shows the results of detection of acrylamide concentration in the sample of Luwak Toraja coffee amounted to 15.56 ppb, with the analysis conditions as shown in table 2.

Table 3 shows the results of acrylamide level measurements in coffee samples electrochemically using CV and LCMS/MS. In the measurements, the two methods were repeated 3 times. The results of measurements of acrylamide levels in coffee samples electrochemically using CV is not significantly different from the results obtained from the LCMS/MS analysis.

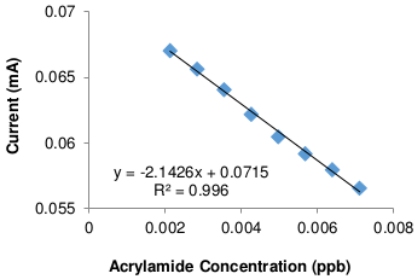


Figure 3. Hb/Pt/BDD electrode calibration curve with standard AA.

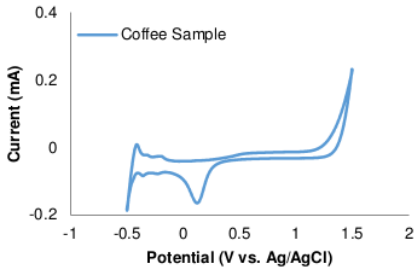


Figure 4. Hb/Pt/BDD electrode voltammogram of acrylamide in Luwak Toraja coffee sample.

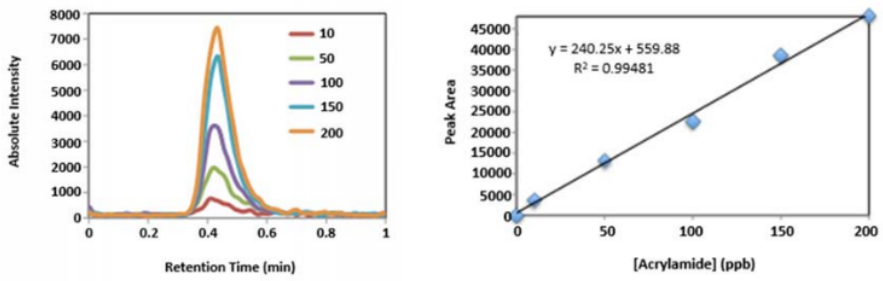


Figure 5. LC-MS/MS in Luwak Toraja coffee samples.

Table 2. Analysis in coffee samples with LC-MS/MS.

Sample	Retention time	Acrylamide 1	Acrylamide 2	Acrylamide 3	Average	Concentration (pbb)
Coffee	0.429	4935	4177	3780	4297.33	15.56

Table 3. Comparison of results obtained by measuring LCMS/MS and CV.

Method	Concentration (pbb)
LCMS/MS	15.56
CV	15.55

4. Conclusion

The Hb/Pt/BDD electrode performance test on the acrylamide standard samples has been proven to be successful with R^2 results of 0.996 and LOD of 0.0015 ppb and standard deviation of 0.00111. The Hb/Pt/BDD electrode was able to detect acrylamide in Luwak Toraja coffee samples 15.55 ppb after multiplied by the dilution factor, in which the results that did not differ significantly when the LC-MS/MS method 15.56 ppb was used. Thsu, it can be concluded that the Hb/Pt/BDD electrode can be used to detect acrylamide in more simple, faster and cheaper manner compared to the LCMS/MS method.

Acknowledgments

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