

Research Article

Sensitive Electrochemical Detection of Glucose at Glucose Oxidase-Cobalt Phthalocyanine-Modified Boron-Doped Diamond Electrode

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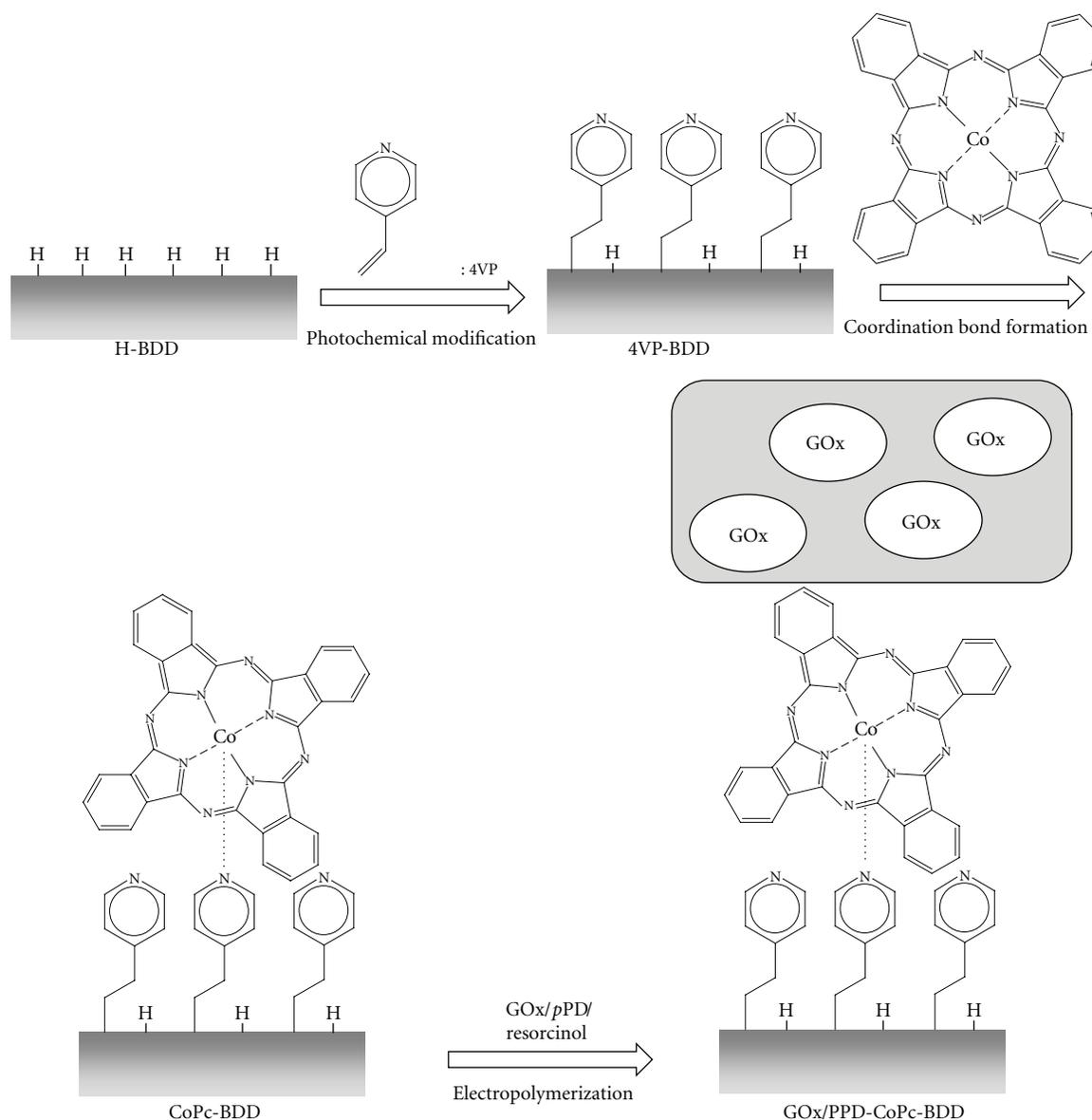
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Electrochemical detection of glucose was achieved at a glucose oxidase (GOx)-cobalt phthalocyanine (CoPc)-modified boron-doped diamond (BDD) electrode without any additional electron mediator in the electrolyte solution. The surface of the hydrogen-terminated BDD thin film prepared by microwave plasma-assisted CVD was modified with 4-vinylpyridine (4VP) via photochemical modification. The 4VP-BDD was then immersed in a CoPc solution to obtain CoPc-BDD. A poly(*p*-phenylenediamine) (PPD) thin film containing GOx was coated on the CoPc-BDD electrode surface via electropolymerization. At the GOx/PPD-CoPc-BDD electrode, anodic current for glucose oxidation was observed with a sigmoidal voltammetric curve, indicating successful electron mediation of H₂O₂ generated as the result of glucose oxidation at GOx. The signal-to-background ratio for voltammetric current of glucose detection was larger at the GOx/PPD-CoPc-BDD electrode than at the GOx/PPD-modified platinum electrode due to the smaller background current of the modified BDD electrode.

1. Introduction

Highly boron-doped diamond (BDD) electrodes have been known to be a promising electrode material for sensitive electroanalysis based on the wide potential window and low background current as well as physical and chemical stability and biocompatibility [1]. Glucose is a compound, whose concentration is one of the most desired to be determined via electrochemical methods in relation to increasing demands for diabetes care [2]. In order to use BDD electrode for glucose detection, one should modify the surface with catalysts or enzymes due to the large overpotential for direct glucose oxidation at unmodified BDD electrodes. For glucose detection at BDD electrodes using electrocatalysts, Cu [3–5] and Ni [6–9] have been used for modification of the surfaces. Immobilization of particles or line patterns of these metals with micrometer or nanometer scale onto BDD surface enables sensitive glucose detection with low background current. On the other hand, BDD electrodes modified with glucose oxidase (GOx) [10–13] are advantageous because

of their selectivity and sensitivity of the glucose molecule. In some cases, additional mediators, such as ferrocene carboxylic acid [11], were employed for glucose detection at GOx-modified BDD electrodes. Glucose detection without any additional reagents to samples is possible for simple analysis systems, and especially for monitoring concentration. Hydrogen peroxide is a product of glucose oxidation at GOx and thus can act as an electroactive mediator for electrochemical detection of glucose. We have reported that sensitive electrochemical detection of hydrogen peroxide can be achieved at BDD electrodes modified with cobalt(II) phthalocyanine (CoPc) [14]. The limit of detection of hydrogen peroxide at CoPc-BDD electrode using a flow-injection system was found to be in the range of 1–10 nM and that was rather low comparing to Pt-modified BDD electrodes (30 nM) [15]. Thus the use of CoPc-BDD should be effective for fabrication of a GOx-modified BDD electrodes for sensitive glucose detection. In the present study, we prepared a GOx containing poly(*p*-phenylenediamine) (PPD) film on a CoPc-BDD electrode surface to fabricate an



SCHEME 1: Fabrication of GOx/PPD-CoPc-BDD electrode.

electrode material for reagentless sensitive glucose detection (Scheme 1). The signal-to-background ratio of glucose was found to be larger at GOx/PPD-CoPc-BDD electrode than at GOx/PPD-Pt electrode.

2. Experimental

BDD electrodes were prepared by microwave plasma-assisted chemical vapor deposition (MPCVD). The deposition condition was identical to what can be found in our previous report [16]. A conductive polycrystalline BDD thin film with a grain size of 1–5 μm was first grown on a conductive silicon wafer substrate. Surface modification of BDD with CoPc was carried out with a procedure described in the previous report [14]. A hydrogen-terminated BDD (H-BDD) sample was immersed in a 100 mM 4-vinylpyridine (4VP)/acetonitrile

solution. The sample surface was then irradiated with UV light (254 nm) from a low pressure mercury lamp (SUV-40, Sen Lights Corp.) in Ar atmosphere through a quartz window for 3 h. The 4VP-modified BDD sample was then immersed in a 0.1 mM CoPc/chloroform solution for one day to obtain CoPc-BDD. Surface modification with 4VP was confirmed with X-ray photoelectron spectroscopy (XPS) with a XP spectrometer (AXIS-NOVA, Kratos). GOx/PPD film was prepared using an electropolymerization method. Potential cycling, repeated 10 times, between 0 to +0.6 V versus Ag/AgCl was performed in 0.1 M phosphate buffer solution (PBS, pH 7) containing 0.15 mM *p*-phenylenediamine, 0.15 mM resorcinol, and 500 U/mL glucose oxidase (Type VII, from aspergillus niger, Sigma-Aldrich) at a CoPc-BDD electrode at a potential sweep rate of 20 mVs⁻¹. For all electrochemical experiments, an

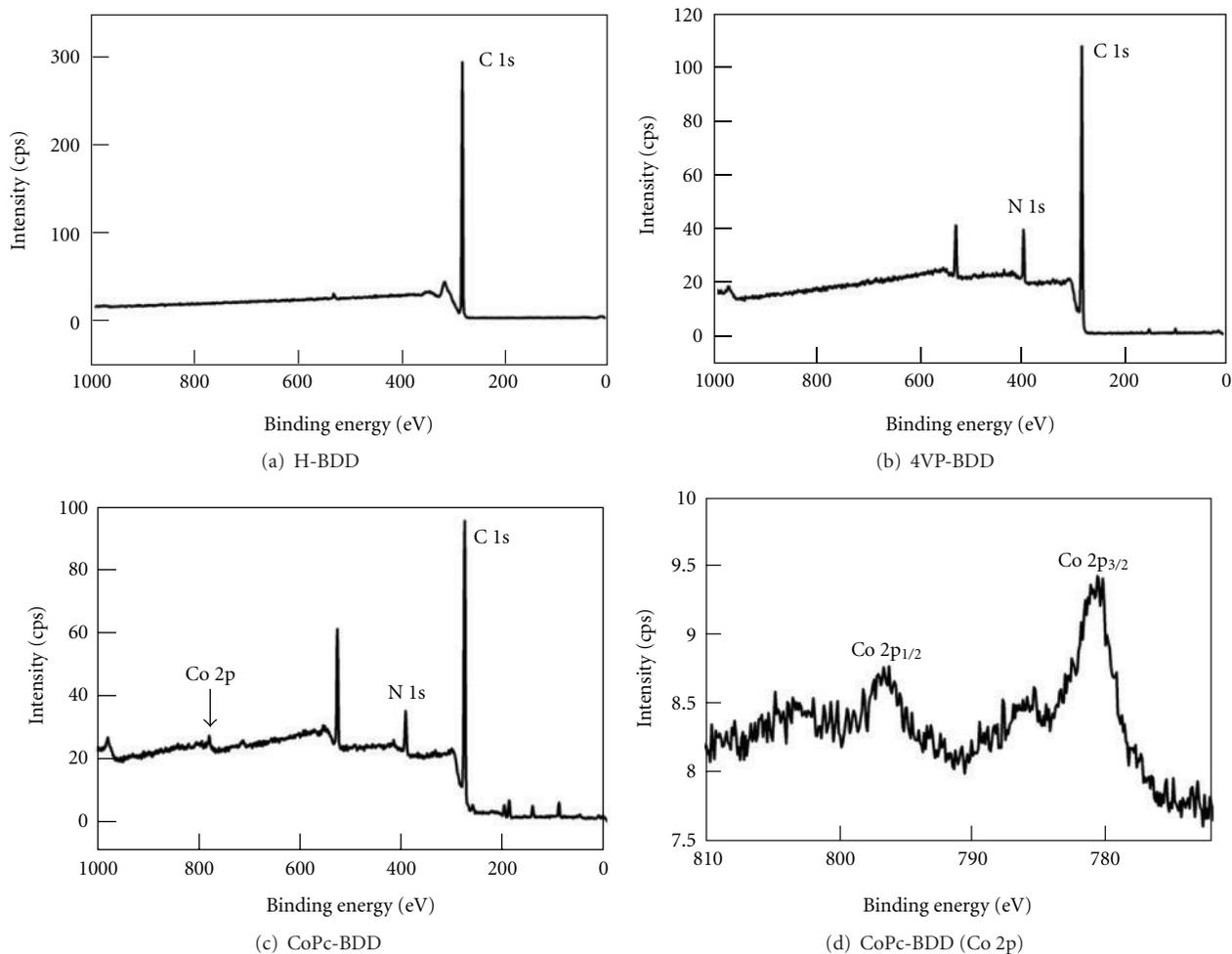


FIGURE 1: XPS of (a) H-, (b) 4VP-, and (c) CoPc-BDD surfaces. (d) Co 2p spectrum of CoPc-BDD surface.

Ag/AgCl electrode with saturated KCl and a platinum spiral wire were used for a reference and a counter electrode, respectively, connecting to a digital potentiostat (HZ-5000, Hokuto Denko).

3. Results and Discussion

3.1. Preparation of CoPc-BDD Electrode. Surface modification of BDD with 4VP was confirmed with XPS. After surface modification with 4VP, an N 1s peak was shown in the XP spectra at 397 eV (Figure 1(b)), which was absent in the spectrum of H-BDD (Figure 1(a)). This should be based on the pyridine moiety on the 4VP-BDD surface. The N/C atomic concentration ratio determined from XPS quantitative analysis was found to saturate with between 2 and 3 h UV irradiation. Thus, we decided the UV irradiation time for the 4VP modification to be 3 h. Immobilization of CoPc on 4VP-BDD surface was also confirmed with Co 2p_{3/2} and (781 eV) Co 2p_{1/2} (797 eV) peaks (Figure 1(c)). The Co/C atomic concentration ratio saturated within 24 h of immersion of a 4VP-BDD in a CoPc solution. Figure 2 shows cyclic voltammograms (CVs) in 0.1 M phosphate

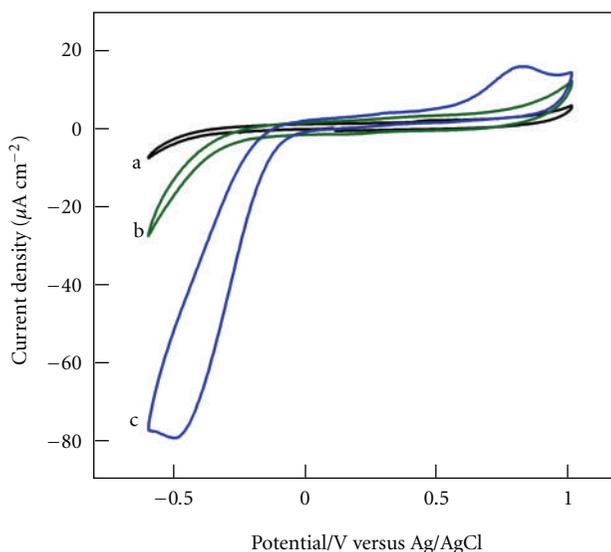


FIGURE 2: CVs in 0.1 M PBS at (a) H-, (b) 4VP-, and (c) CoPc-BDD electrodes. Potential sweep rate was 100 mVs⁻¹.

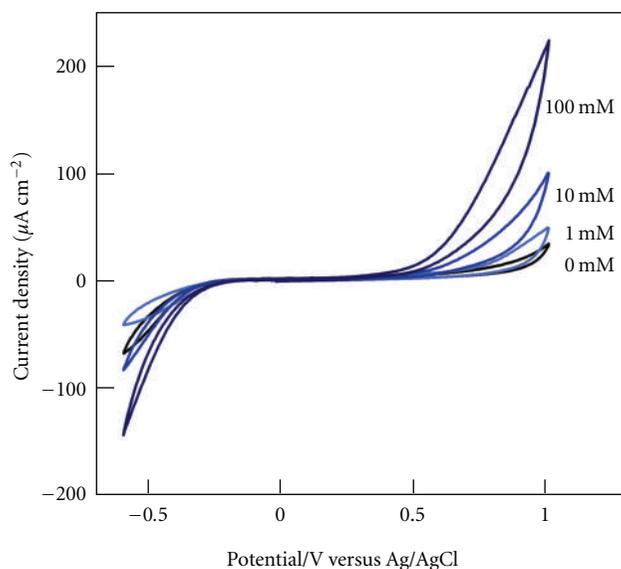


FIGURE 3: CVs in 0.1 M PBS containing 0, 1, 10, and 100 mM H_2O_2 at CoPc-BDD electrode. Potential sweep rate was 100 mVs^{-1} .

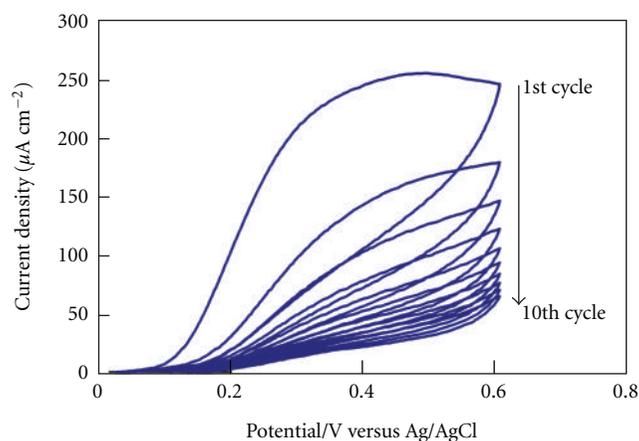


FIGURE 4: CVs in 0.1 M PBS containing 0.15 mM *p*-phenylenediamine, 0.15 mM resorcinol and 500 U/mL GOx at CoPc-BDD electrode for electropolymerization of a GOx/PPD film. Ten successive cycles are indicated. Potential sweep rate was 20 mVs^{-1} .

buffer solution (PBS, pH 7) at modified BDD electrodes. Although no redox peak was found in the CVs at H- and 4VP-BDD electrode, an anodic and a cathodic peak was found at the CoPc-BDD electrode. This result indicates that the immobilized CoPc was redox-active on the modified electrode [14]. Figure 3 shows CVs at CoPc-BDD electrode in 0.1 M PBS containing various concentrations of H_2O_2 . At unmodified (H-terminated) BDD surface, no increase of the faradaic current for H_2O_2 was found. This is due to a large overpotential of the electrode reaction of H_2O_2 at the unmodified BDD electrode. On the other hand, the CV at CoPc-BDD showed an anodic and cathodic faradaic current depending on H_2O_2 concentration, based on the

electrocatalytic activity of CoPc to the electrode reaction of H_2O_2 [17].

3.2. Preparation of GOx/PPD-CoPc-BDD Electrode. In order to immobilize GOx onto the electrode surface, GOx/PPD film was formed via electropolymerization method. Figure 4 shows ten successive CVs for the electropolymerization in 0.1 M PBS containing 0.15 mM *p*-phenylenediamine, 0.15 mM resorcinol and 500 U/mL GOx at CoPc-BDD electrode. The decrease in the anodic current is typical of electropolymerization of similar polymer films and is based on the insulating properties of the film formed at the interface [18]. Figure 5 shows scanning electron microscopy (SEM) images of a BDD and a GOx/PPD-CoPc-BDD electrode surfaces. PPD is known to be a stiff polymer with a ladderlike network structure [19], and it should be responsible for the linear texture of the film observed with SEM. In addition, the PPD film formed with electropolymerization method is known to be a permselective membrane for H_2O_2 [19] and can suppress electrode reaction of electroactive interferences such as ascorbic acid and dopamine [14]. Thus, the use of PPD matrices should be advantageous for selective detection of glucose.

3.3. Glucose Detection. Figure 6(a) shows a CV in 0.1 M PBS containing 5 mM glucose at a GOx/PPD-CoPc-BDD electrode. In the absence of glucose, almost no current was observed. However, in the presence of glucose, anodic current began to flow at +0.15 V versus Ag/AgCl and was almost constant at +0.3 V versus Ag/AgCl or more positive potentials. This sigmoidal curve is typical of enzyme electrode reactions [11], indicating successful electron transfer from glucose to the BDD electrode. In the case of an electrode with a GOx/PPD film formed on the H-BDD surface (GOx/PPD-H-BDD), no current response was observed in the presence of glucose in the electrolyte solution (Figure 6(b)). These results indicate that the current response observed for GOx/PPD-CoPc-BDD was not with direct electron transfer from glucose or GOx and should be based on the mediation of H_2O_2 electrooxidation catalyzed by the CoPc on the BDD surface. Thus, it was shown that electrocatalysts, such as CoPc, are essential for the current response of BDD-based enzyme electrodes using oxidases that can generate H_2O_2 . Similar to the case of GOx/PPD-CoPc-BDD electrode, a sigmoid-shaped voltammetric curve was shown at a Pt electrode covered with a GOx/PPD film via electropolymerization (GOx/PPD-Pt, Figure 6(c)). This result was not surprising because H_2O_2 can be oxidized on a Pt electrode at low potentials without the use of any additional catalyst. The signal current at +0.5 V versus Ag/AgCl was larger at GOx/PPD-Pt electrode ($54 \mu\text{A cm}^{-2}$) than at GOx/PPD-CoPc-BDD electrode ($25 \mu\text{A cm}^{-2}$). On the other hand, however, the background current was much lower at GOx/PPD-CoPc-BDD electrode ($1.8 \mu\text{A cm}^{-2}$) than at GOx/PPD-Pt electrode ($14 \mu\text{A cm}^{-2}$). As a result, the signal-to-background (S/B) ratio at this potential was found to be 12.5 at the GOx/PPD-CoPc-BDD electrode while that was 3.8 at the GOx/PPD-Pt electrode. As seen in previous reports, low background

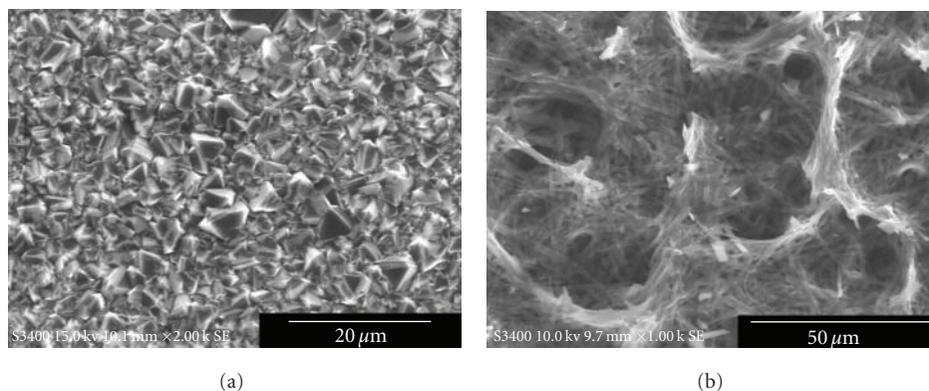


FIGURE 5: SEM image of (a) unmodified BDD and (b) GOx/PPD-CoPc-BDD surface.

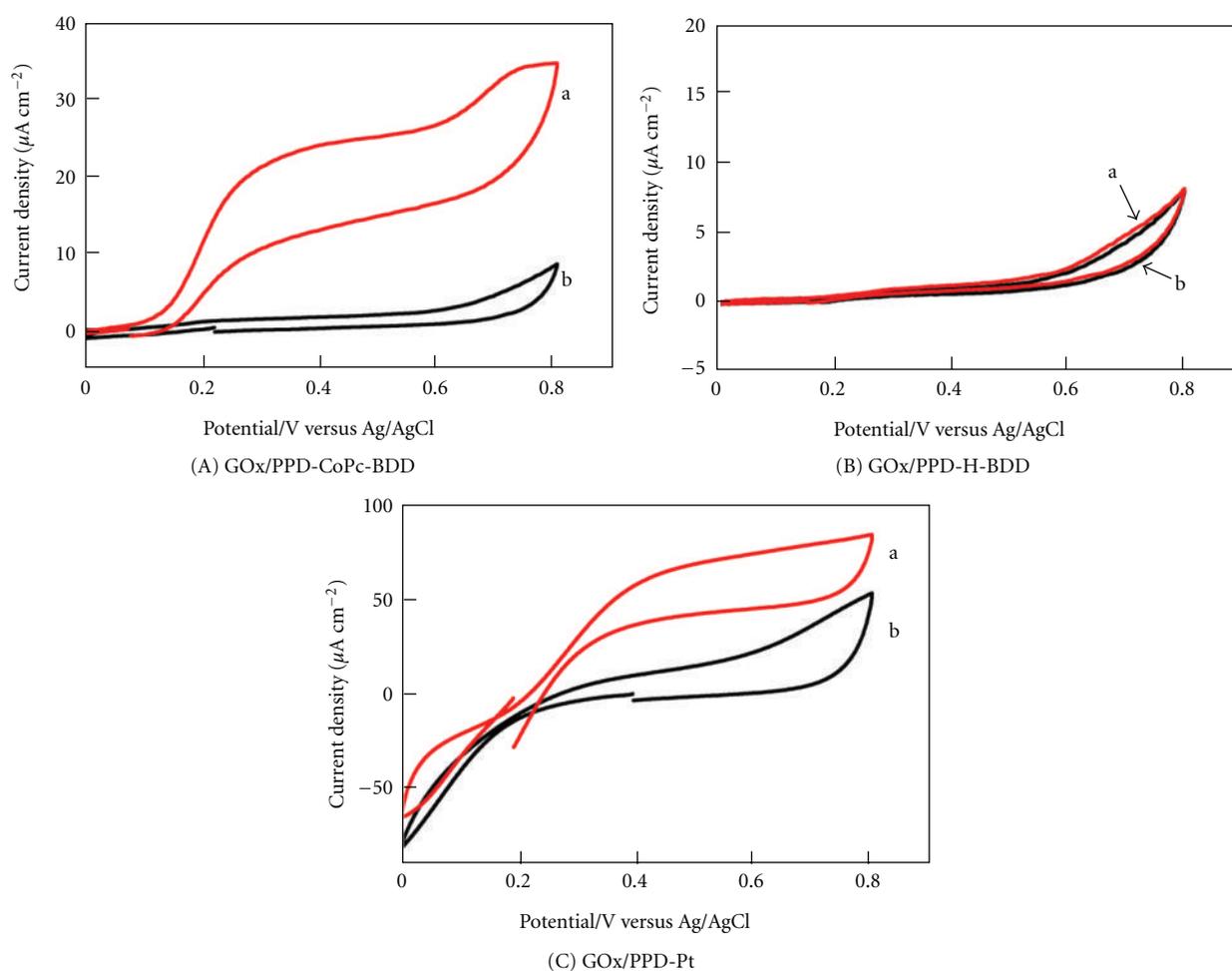


FIGURE 6: CVs for 5 mM glucose in 0.1 M PBS at (A) GOx/PPD-CoPc-BDD, (B) GOx/PPD-H-BDD, and (C) GOx/PPD-Pt electrodes. Curves (a) and (b) indicate CVs for 5 mM glucose and background, respectively. Potential sweep rate was 5 mVs^{-1} .

current at modified BDD electrodes enabled sensitive electrochemical detection of oxalic acid [20] and H_2O_2 [14]. Also in this study, the low background current feature of BDD was maintained even after surface modification with 4VP, CoPc and GOx/PPD film. Therefore, immobilization of enzymes

on CoPc-BDD should be a useful method for fabrication of sensitive enzyme electrodes using other types of oxidase which can generate H_2O_2 (e.g., uricase, cholesterol oxidase, lactate oxidase, alcohol oxidase, bilirubin oxidase, or choline oxidase) based on the low background current.

4. Conclusion

GOx-immobilized PPD polymer film was formed on a CoPc-BDD electrode via electropolymerization. At the GOx/PPD-CoPc-BDD electrode, CV for glucose showed a sigmoidal curve without any additional electron mediator in the electrolyte solution, indicating that the glucose oxidation at the GOx and the electrocatalytic oxidation of H₂O₂, which is a product of glucose oxidation, at the CoPc were successful at the electrolyte/electrode interface. Since the GOx/PPD-CoPc-BDD electrode exhibited significantly small background current, the S/B ratio was found to be larger at the GOx/PPD-CoPc-BDD electrode than at the GOx/PPD-Pt electrode. This result is consistent with our previous result that H₂O₂ can be detected electrochemically on the CoPc-BDD electrode with the lowest limit of detection level based on the small background current [14]. This study showed that this feature of the CoPc-BDD electrode can be maintained even after further modification with GOx. Therefore, sensitive enzyme electrodes, involved with H₂O₂/O₂ redox mediation, having low limit of detection should be able to be fabricated with a similar method using a CoPc-BDD electrode. For application of this electrode to a glucose sensor, amperometric studies using a solution containing glucose and other possible interferents which exist in human blood should be investigated in the future study.

Acknowledgments

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