Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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Published online: 11 Sep 2013.

To cite this article: Haiming Zhao, Seong Hun Yu, Pil J. Yoo, Jae Hyung Park & Jun Young Lee (2013) Glucose Sensing by Glucose Oxidase/PEDOT Thin Film Electrode, Molecular Crystals and Liquid Crystals, 580:1, 22-28, DOI: 10.1080/15421406.2013.803898

To link to this article: http://dx.doi.org/10.1080/15421406.2013.803898

Mol. Cryst. Liq. Cryst., Vol. 580: pp. 22–28, 2013

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2013.803898

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Glucose Sensing by Glucose Oxidase/PEDOT Thin Film Electrode

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Glucose oxidase (GOX)/poly(3,4-ethylenedioxythiophene) (PEDOT) electrode was prepared for glucose sensing. PEDOT thin film was first electrochemically or chemically polymerized on Pt substrate, followed by dipping it into GOX solution to immobilize GOX on PEDOT surface. It was observed that GOX was adsorbed much more efficiently on the chemically polymerized PEDOT than on the electrochemically polymerized PEDOT. The cyclic voltammetry of the thin film electrode showed a clear anodic peak at −0.43 V. It was also observed that the anodic current monotonically decreased with the increase of glucose concentration dissolved in the electrolyte solution, implying that the electrode can be applied as a simple glucose sensor.

Keywords Direct electron transfer; enzyme electrode; glucose oxidase; glucose sensing; PEDOT

1. Introduction

Glucose oxidase (GOX) is known as a typical flavin enzyme, where FAD (flavin adenine dinucleotide, oxidized state) and $FADH_2$ (reduced state) are incorporated as the active sites for redox reaction. Recently, direct electron transfer of GOX has been intensively studied for enzymatic sensing of glucose and biofuel cells [1,2]. For biosensing of glucose, electrochemical method has been widely used because of low cost and high sensitivity [3–6]. To prepare enzymatic sensing electrode, GOX was immobilized on the modified metal electrodes with nano-metal oxide materials or carbon nanotube [7–10]. However, most of the GOX immobilized electrode systems required redox mediators such as ferrocenemethanol [11,12].

Poly(3,4-ethylenedioxythiophene) (PEDOT) is considered as the promising conducting polymer with high conductivity for many applications including solar cell [13], super capacitor [14] and glucose biosensor [15]. Electrochemically polymerized PEDOT thin film was used as glucose biosensor [16], but direct electron transfer of GOX was not taken account in that research. Some studies were also reported to incorporate GOX more efficiently into PEDOT, but all the electrochemical signals could be obtained only with

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redox mediators. Direct electron transfer of GOX using vapour polymerized PEDOT was achieved, in which sensing characterics of glucose was not reported [17].

In this paper, we report a simple process to fabricate GOX/PEDOT enzymatic electrode and its glucose sensing behaviour through the direct electron transfer of the electrode. Enzyme electrode was prepared by two steps. PEDOT thin film was first chemically or electrochemically polymerized on Pt substrate, followed by dipping it into GOX solution to immobilize GOX on PEDOT surface. Electrochemical properties and glucose sensing behaviour of the electrode were studied using cyclic voltammetry.

2. Experimental

2.1 Preparation and Characterization of GOX/PEDOT Enzyme Electrode

Synthesis of PEDOT thin film on Pt.

Electrochemical synthesis. Since electrochemical growth of PEDOT may be complicated due to poor solubility of EDOT monomer in aqueous solution, poly(ethylene glycol) (PEG, MW = 10,000 g/mol) was used to improve the solubility of the monomer [16]. Polymerization solution was prepared by dissolving 0.01 mM of 3,4-ethylenedioxythiophene (EDOT) (Clevios^{TM} MV2) and 0.25 mM of PEG in 0.02 M phosphate buffer saline (PBS) solution (pH 7.4) as the supporting electrolyte solution. The polymerization solution was degassed by vigorous nitrogen bubbling for 30 minutes before polymerization. Electrochemical polymerization was carried out by cyclic voltammetric (CV) method using VSP Potentiostat (Princeton Applied Research) between +0.2 and +1.2 V versus Ag/AgCl (saturated KCl) reference electrode at the rate of 100 mV/s for 10 cycles, producing PEDOT thin film (electrochem-PEDOT) on the Pt electrode with the thickness of about 30 nm.

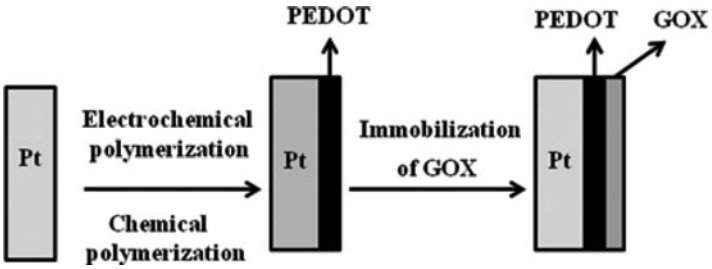
Chemical synthesis. PEDOT thin film was fabricated using EDOT as a monomer and ferric p-toluenesulfonate (FTS) as an oxidant via in-situ polymerization. Typical chemical polymerization procedure is as following. Monomer solution was prepared by dissolving 1.4 mmol of EDOT, 0.02 g of poly(vinyl pyrrolidone) (Sigma Aldrich, MW = 360,000 g/mol) and 1.4 mmol of pyridine (Sigma Aldrich, 99.8%) in 2 ml of 1-butanol (Sigma Aldrich, 99.8%). FTS solution (Clevios^{TM}, CE 40) was used as the oxidant solution as received. The monomer and oxidant solutions were then mixed, where mole ratio of FTS to EDOT was 1:1.5. The mixed solution was spin-coated on the Pt electrode with 1 cm^2 area at 2,000 rpm for 30 seconds and polymerized at 70°C for 20 minutes. The resulting PEDOT film was washed with methanol to remove residual oxidant, monomer and oligomer and finally dried in a convection oven, producing PEDOT thin film (chem-PEDOT) with the thickness of about 150 nm.

Immobilization of GOX on PEDOT Thin Film.

To immobilize GOX on PEDOT film, PEDOT/Pt electrode was immersed into GOX (Sigma Aldrich) aqueous solution (1,000 UI/ml) for 60 minutes at 25°C. After immobilization process, the electrode was washed with methanol and deionized water to remove excess GOX and impurity on PEDOT surface and then finally dried in a convection oven. Preparation procedure for GOX/PEDOT/Pt electrode is shown in Scheme 1.

Characterization of GOX/PEDOT Electrode.

Electrical conductivity of PEDOT thin film was measured by four point probe (Dong Ah Trade & Tech Corp.) connected with Fluke 45 Dual Display Multimeter (Fluke Corporation) and Yokogawa 2553 Single Output DC Supply (Yokogawa Electric Co., Ltd.). Thickness of the film was measured by Alpha-step IQ surface profiler (KLA



Scheme 1. Fabrication process for GOX/PEDOT/Pt enzyme electrode.

Tencor). The surface morphology and root-mean-square (RMS) roughness were studied by AtomicForceMicroscopy(AFM) (SPA-300HV).

2.2 Electrochemical Properties and Glucose Sensing of GOX/PEDOT/Pt Electrode

Electrochemical properties of GOX/PEDOT electrode were studied by CV using a VSP Potentiostat, where 0.02 M PBS solution (pH 7.4) and Pt plate were used as the electrolyte solution and the counter electrode, respectively. Voltage was scanned between −0.7 V and −0.1 V versus Ag/AgCl (saturated KCl) reference electrode at the scan rate of 10 mV/s. In order to investigate glucose sensing behaviour of the GOX/PEDOT/Pt electrode, CV was carried out in the 0.02 M PBS solution (pH 7.4) containing various amount of D-glucose.

3. Results and Discussion

3.1 Improvement of Electrical Conductivity

Electrical conductivity of PEDOT is one of the most important properties for efficient electron transfer of the electrode. Since it was reported that pyridine acted as an inhibitor during chemical polymerization of PEDOT [18], we investigated the effect of the concentration of pyridine on the conductivity of chemically polymerized PEDOT. As shown in Fig. 1, the conductivity linearly increased with the concentration of pyridine, showing the

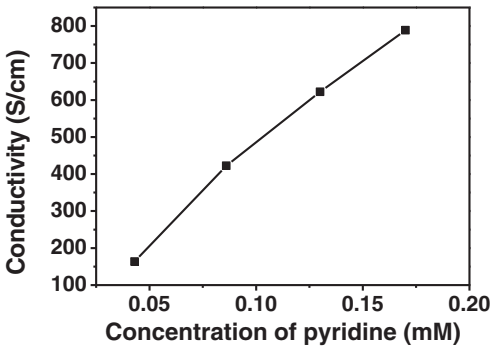


Figure 1. Electrical conductivity change of chem-PEDOT thin film with the concentration of pyridine.

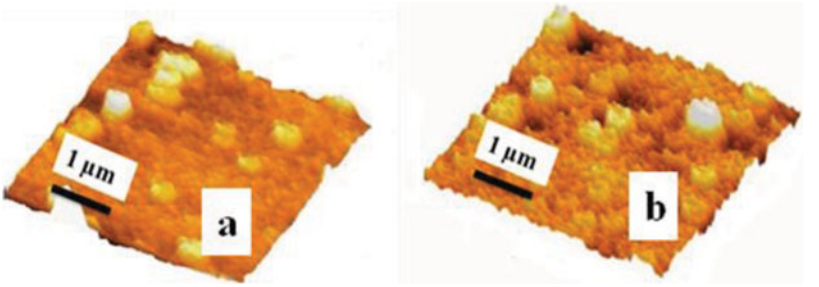


Figure 2. AFM images of (a) chem-PEDOT/Pt and (b) GOX/chem-PEDOT/Pt electrode surfaces.

highest conductivity of 790 S/cm at 0.17 mM of pyridine concentration. Pyridine makes the reduction of Fe^{3+} to Fe^{2+} more difficult to oxidize EDOT monomer [18], resulting in slow polymerization rate. The controlled polymerization rate with pyridine may produce higher molecular weight of PEDOT and more expanded conformation, thus, the higher conductivity as shown in Fig. 1. However, polymerization did not take place with use of excess pyridine because pyridine suppressed the polymerization too much. Therefore, PEDOT thin film could not be obtained with the concentration of pyridine over 0.17 mM. The electrical conductivity of the chem-PEDOT was quite stable under ambient condition.

3.2 Electrochemical Properties of GOX/PEDOT Electrode

It is expected that the electrostatic interaction between negatively charged GOX and positively charged PEDOT can effectively immobilize GOX on PEDOT surface. It was found that GOX was more efficiently immobilized on the chem-PEDOT than on the electrochem-PEDOT. More efficient adsorption of GOX on chem-PEDOT could be confirmed by the thickness change of the electrode before and after GOX immobilization process. After GOX immobilization, thickness of the chem-PEDOT film increased by about 60% from 150 nm to 240 nm, while thickness of the electrochem-PEDOT increased by only about 14% from 32 nm to 37 nm. This confirms much more efficient immobilization of GOX on chem-PEDOT. It was also observed that surface roughness of chem-PEDOT film changed little from 3.0 to 4.1 nm after GOX immobilization as shown in Fig. 2, indicating uniform immobilization of GOX on chem-PEDOT surface.

Figure 3 displays the cyclic voltammograms of GOX/electrochem-PEDOT and GOX/chem-PEDOT electrodes, showing the clear anodic current. Even though the active redox parts (FAD and $FADH_2$) of GOX are deeply located inside the insulative apoenzyme [19–20], GOX of this system could be electrochemically oxidized during the voltage scan in the forward direction by following reaction shown in Equation (1).

$$GOX(FADH_2) \rightarrow GOX(FAD) + 2H^+ + 2e^- \quad (1)$$

And when oxygen exists around the electrode in the solution, oxygen can be chemically reduced by GOX as shown in Equation (2) [10].

$$GOX(FADH_2) + O2 \rightarrow GOX(FAD) + H_2O_2 \quad (2)$$

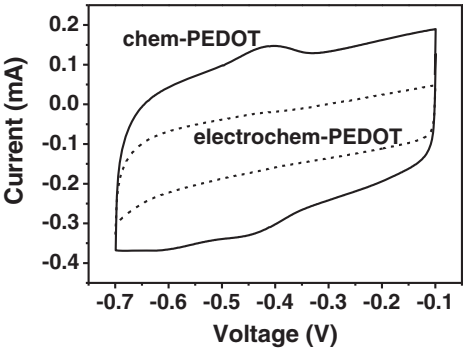


Figure 3. Cyclic voltammograms of GOX/chem-PEDOT/Pt and GOX/electrochem-PEDOT/Pt electrodes in 0.02 M PBS solution at the sweep rate of 10 mV/s.

Therefore, combining Equations (1) and (2) produces the net reaction as Equation (3) when oxygen exists around the electrode during applying voltage in the forward direction [17].

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \tag{3}$$

As shown in Fig. 3, GOX/chem-PEDOT/Pt electrode exhibited a clear anodic current at −0.43 V, confirming the direct electron transfer through the electrode. However, redox peaks of GOX/electrochem-PEDOT/Pt electrode were too weak to be observed as shown in Fig. 3, meaning a little amount of GOX was adsorbed on PEDOT surface. This also confirms more efficient adsorption of GOX on chem-PEDOT surface.

Electrochemical activity of GOX/chem-PEDOT decreased with repeated cycles as shown in Fig. 4. It is considered that GOX layers far from PEDOT surface are weakly bound to PEDOT because of weaker electrostatic interaction. This weak binding between GOX and PEDOT possibly resulted in desorption of GOX from PEDOT surface upon cycling.

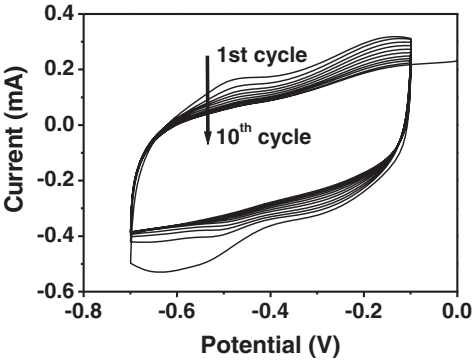


Figure 4. Repeated cyclic voltammograms of GOX/chem-PEDOT/Pt electrode in 0.02 M PBS solution at the sweep rate of 10 mV/s.

3.3 Glucose Sensing Behavior of GOX/chem-PEDOT/Pt Electrode

In the presence of glucose, the glucose can be chemically oxidized to gluconolacton by GOX(FAD) as shown in Equation (4).

$$\text{Glucose} + \text{GOX(FAD)} \rightarrow \text{Gluconolacton} + \text{GOX(FADH}_2\text{)} \tag{4}$$

Combining Equations (2) and (4) resulted in the net reaction (5), where glucose reduces oxygen to H_2O_2 with the catalysis of GOX.

$$\text{Glucose} + \text{O}_2 \xrightarrow{\text{GOX}} \text{Gluconolacton} + \text{H}_2\text{O}_2 \tag{5}$$

Therefore, the concentration of oxygen around the electrode decreases with the increase of glucose concentration. This decrease in the concentration of oxygen would lead to the decrease of the anodic current shown in Equation (3).

Figure 5(a) shows CV curves of GOX/chem-PEDOT electrode in PBS solution containing various concentrations (0, 5, 10, 15 and 20 mM) of glucose. The reduction current of oxygen at −0.46 V in Fig. 5(a) decreases monotonically from 0.17 to 0.08 mA with the increase of glucose concentration as shown in Fig. 5(b) as the described reaction mechanism. This implies the possibility of GOX/chem-PEDOT enzyme electrode for glucose sensor through the direct electron transfer mechanism without any redox mediator.

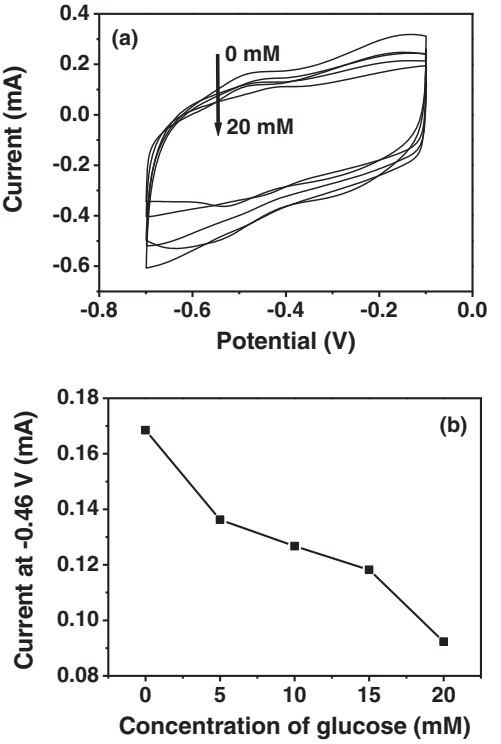


Figure 5. (a) Cyclic voltammograms of GOX/chem-PEDOT/Pt in 0.02 M PBS solution containing various amounts of glucose at the sweep rate of 10 mV/s and (b) the current values at −0.46 V.

4. Conclusions

We fabricated GOX/PEDOT enzyme electrode for glucose sensing. PEDOT thin film was first chemically polymerized on Pt substrate, followed by immobilization of GOX on PEDOT film by dipping PEDOT film into GOX solution. The direct electron transfer through the electrode was confirmed by the reduction peak of oxygen at −0.43 V. It was also observed that the reduction current of oxygen at −0.46 V decreased monotonically with the increase of glucose concentration dissolved in the electrolyte solution, implying that the electrode can be applied as the simple glucose sensor.

Acknowledgment

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) grants funded by the Korea government (Ministry of Education, Science and Technology) (2010-0029410, 2010-0026281, 2010-0010643).

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