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Enhancement of Glucose Sensing Behavior of Cobalt Tetraphenylporphyrin Thin Film Using Single-Wall Carbon Nanotubes

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We have developed a glucose biosensor based on single-wall carbon nanotubes (SWCNTs) and cobalt tetraphenylporphyrin (CoTPP) thin film using glucose oxidase (GOx). In this work, a plasma-polymerized thin film of CoTPP was prepared on indium tin oxide (ITO) electrodes and we examined the electrochemical biosensing ability of GOx-immobilized CoTPP/SWCNT/CoTPP/ITO electrodes towards glucose. Cyclic voltammetric (CV) methods were used to investigate the electrochemical behavior of these electrodes. The effect of radio frequency (rf) power of plasma for the deposition of the CoTPP polymer on glucose sensing was also studied, and it was found that the sensitivity of these devices increases with decreasing plasma power. Our glucose biosensor based on CNTs shows high sensitivity towards glucose, which promises the application of these devices as glucose biosensors.

1. Introduction

The development of an effective biosensor for the detection of glucose is very important for the clinical diagnosis of diabetes and in the food industry for quality control. Several techniques have been developed for glucose sensing such as optical (fluorescent),^(1,2) electromagnetic (using microwaves),⁽³⁾ quartz crystal microbalance (QCM),⁽⁴⁾ and electrochemical assays.^(5,6) Electrochemical detection offers several advantages including low cost, reliability, high sensitivity, selectivity, and simplicity. Research efforts exploring the advantages of this detection assay have already been reported.⁽⁷⁾

Scientific investigations on amperometric biosensors employing carbon nanotubes (CNTs) have already been carried out.^(8,9) The unique properties of CNTs such as high electrical conductivity, large surface area due to their nanometer size, and mechanical

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strength make them promising for biosensing applications. In electrochemical reactions, CNTs have the ability to promote electron transfer when used as electrode materials. For the application of CNTs in biosensors, the attachment of CNTs to biomolecules (enzymes) and electrodes is needed. The lack of solubility of CNTs in most solvents is the major barrier for the attachment of biomolecules to CNTs. The method of chemically functionalizing CNTs⁽¹⁰⁾ has made it possible to modify CNTs with biomolecules. The major disadvantage of this covalent functionalization is that the intrinsic properties of CNTs will be damaged during modification. Another technique is the use of mediators such as N,N-dimethyl formamide (DMF),⁽¹¹⁾ Nafion,⁽¹²⁾ chitosan,⁽¹³⁾ poly(diallyldimethyl ammonium chloride) (PDDA),⁽¹⁴⁾ Teflon,⁽¹⁵⁾ polyethyleneimine,⁽¹⁶⁾ electropolymerized films of poly-*o*-aminophenol,^(17,18) polypyrrole,⁽¹⁹⁾ polyaniline,⁽²⁰⁾ 3,3'-diaminobenzidine (DAB)⁽²¹⁾ and poly(toluidine blue O).⁽²²⁾ In the case of these polymers, the preparation of a homogenous, reproducible, strongly adhesive thin film (< 1 μm) to substrates is very difficult because they are fabricated by spin- and dip-coating methods.

Porphyrins have received a great deal of research interest owing to their crucial role in living systems. They can bind to transition metals (Co, Zn, Mn, Ni etc.) to form porphyrin complexes or metallated porphyrins. The physical and chemical properties of metalloporphyrin complexes make them good gas sensing materials for detecting gaseous substances such as HCl,⁽²³⁾ NO_x,⁽²⁴⁾ and volatile organic compounds such as alcohols⁽²⁵⁾ and amines.⁽²⁶⁾ A thin film of cobalt tetraphenylporphyrin (CoTPP) has been used as a sensing element for the detection of vapors of methyl, ethyl, and isopropyl alcohols⁽²⁷⁾ and cyclohexane.⁽²⁸⁾

Plasma-polymerized thin films formed in plasma in the vapor phase provide an attractive method to be used for the interfacial design of biosensors.⁽²⁹⁾ These films are formed directly on the electrode surface in a glow discharge. The resulting films are extremely thin (< 1 μm), form a homogenous, flat surface, and have good adhesion to the substrate. The surface of these plasma-polymerized films has good affinity for biological components and thus offers a suitable interface between biomolecules and electrodes.⁽³⁰⁾ They are pin-hole free and are chemically and mechanically stable because of their highly branched and cross-linked structure. Moreover, these films can act as antifouling coating against interferent materials such as ascorbic acid and acetaminophen. Hexamethyldisiloxane^(30,31) and acetonitrile^(32,33) are widely used for the preparation of plasma-polymerized thin films for the development of glucose biosensors.

Our group had already proposed an amperometric glucose biosensor based on plasma-polymerized CoTPP film on ITO electrodes.⁽³⁴⁾ In order to examine the role of CNTs in glucose sensing processes and as an improvement of the above work, we attempted to develop a glucose biosensor based on CNTs and CoTPP plasma-polymerized thin film on optically transparent ITO electrodes. We chose SWCNTs from the CNT family and GOx for the construction of the biosensor. We also examined the effect of plasma power for the deposition of the CoTPP polymer film on glucose sensing. The preparation of a CoTPP thin film on ITO electrodes, fabrication of SWCNT-based electrodes, and the electrochemical characteristics of GOx-immobilized CoTPP/ITO electrodes with and without SWCNTs are discussed in detail in the following sections.

2. Experimental Methods

2.1 Chemicals and reagents

Glucose oxidase (GOx) from *Aspergillus niger* (E.C.1.1.3.4, type VII, 50,000 units/g) and 5,10,15,20-tetraphenyl-21H,23H-porphine cobalt (II) were purchased from Sigma Aldrich. SWCNTs were purchased from Sigma (diameter: 1.1 nm, length: 0.5–100 μm). d-Glucose, ethanol, and distilled water were purchased from Kanto Chemical Inc. Japan. Phosphate-buffered saline (PBS) was purchased from Nacalai Tesque, Inc. Japan. All the chemicals and solvents used in this work were used without further purification.

2.2 Preparation of CoTPP thin film on ITO

ITO sheets (10 \times 10 cm²) were cut into small pieces of 2 \times 1 cm² size and cleaned ultrasonically with acetone, ethanol, and distilled water before the deposition of the polymer thin film. The CoTPP polymer thin film on ITO substrates was prepared in accordance with our previous report.⁽³⁴⁾ A detailed description of the apparatus for the plasma polymerization of CoTPP on an ITO electrode has already been published.⁽³⁴⁾ In short, the plasma system consists of a Pyrex glass bell jar, a rotary pump, a heater, a pair of parallel electrodes, and a 13.56 MHz radio frequency (rf) generator through an impedance-matching circuit in order to minimize the reflected power. Its lower electrode is connected to the heater and its upper one to the radio frequency generator. The matching box introduces rf power into the reactor in the range of 10 to 100 W. CoTPP powder was placed on the lower electrode and ITO (1 cm of ITO is masked in order to prevent CoTPP coating) on the upper electrode. A shutter was placed between the lower and upper electrodes, which prevents the excess deposition of CoTPP. Then, the chamber was evacuated to 5–6 Pa (shutter closed) and the lower electrode was heated to 390°C. At 380°C, the shutter was opened so that the evaporated monomer was deposited on the ITO substrate. This evaporated CoTPP shows very weak adhesion to the ITO substrate and is highly soluble in water. The polymer thin films produced in the presence of plasma are insoluble in organic solvents and can act as a protective layer on evaporated polymer films. In order to obtain a protective thin layer over this vacuum-deposited polymer film, plasma was discharged to form a plasma-polymerized thin film of CoTPP on ITO substrates at 390°C. We have used different plasma powers of 30 and 50 W, an exposure time of 1 min, 5 Pa pressure, and 5 mL/min flow rate for polymer thin-film deposition.

2.3 Fabrication of GOx/CoTPP/ITO electrodes

The polymer CoTPP was deposited on ITO electrodes with the aid of plasma polymerization. These plasma-polymerized CoTPP-coated ITO electrodes were immersed in GOx solution (250 mg in 5 mL of distilled water) for about 30 min at 30°C. This introduces GOx enzyme on the surface of CoTPP/ITO substrates resulting in the formation of GOx/CoTPP/ITO electrodes. The device was then rinsed thoroughly with distilled water and stored at 4°C in PBS.

2.4 Fabrication of GOx/CoTPP/SWCNT/CoTPP/ITO electrodes

SWCNTs were dispersed in a 1:1 mixture of ethanol and PBS and these suspended SWCNTs were dropped onto the CoTPP/ITO substrate to form SWCNT/CoTPP/ITO electrodes. A very thin layer of CoTPP film (exposure time: 45 s) was coated on these electrodes as a protective layer to ensure the adhesion of SWCNTs on CoTPP/ITO electrodes.

The GOx solution (250 mg in 5 mL of distilled water) was then drop-casted onto these electrodes to introduce GOx enzyme on the surface of these electrodes. The device was rinsed thrice with distilled water and stored at 4°C in PBS.

2.5 Measurements

Electrochemical experiments were performed with an electrochemical analyzer (ALS/chi, Model 601 A) at room temperature. A standard three-electrode voltammetric system was used with Ag/AgCl electrode as the reference electrode, platinum wire (purchased from Nilaco Corporation, Tokyo, Japan) as the counter electrode, and an enzyme electrode (GOx/CoTPP/ITO and GOx/CoTPP/SWCNT/CoTPP/ITO) as the working electrode (1 cm²). Cyclic voltammetric measurements were carried out in 0.1 M PBS (pH 7.0). A magnetic stirrer was used to stir the solutions during the experiments.

Scanning electron microscopy (SEM, JEOL, JSM-7400F) was carried out at an acceleration voltage of 5 kV in order to study the surface morphology of the devices. The thickness of the polymer thin films on ITO substrates with different rf powers was also studied using this instrument.

3. Results and Discussion

3.1 Surface morphology studies

A thin film of CoTPP was deposited on ITO substrates using plasma polymerization with rf powers of 30 and 50 W. The thicknesses of the obtained thin films were in the range of 200–230 and 500–520 nm for 30 and 50 W, respectively [Figs. 1(a) and 1(b)]. The surface morphology of plasma-polymerized CoTPP thin films (30 and 50 W) on ITO

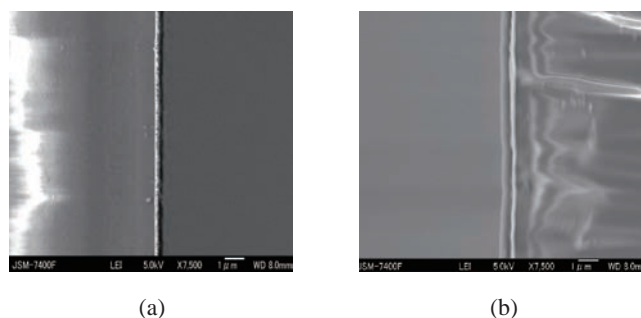


Fig. 1. Cross-sectional SEM view of CoTPP-coated ITO substrate. Thickness of CoTPP coating over ITO substrate at (a) 30 W and (b) 50 W.

is shown in Figs. 2(a) and 2(b). The image clearly shows the pin-hole-free, homogenous surface of the CoTPP film on ITO substrates. A SEM image of the CoTPP/ITO device after dropping the enzyme GOx is shown in Fig. 2(c). Figure 2(d) shows the SEM image of the device surface after drop-casting SWCNTs. It shows the distribution of SWCNTs over the plasma-polymerized thin film on ITO electrodes. Figure 2(e) shows an image of CoTPP coating over the SWCNT/CoTPP/ITO devices.

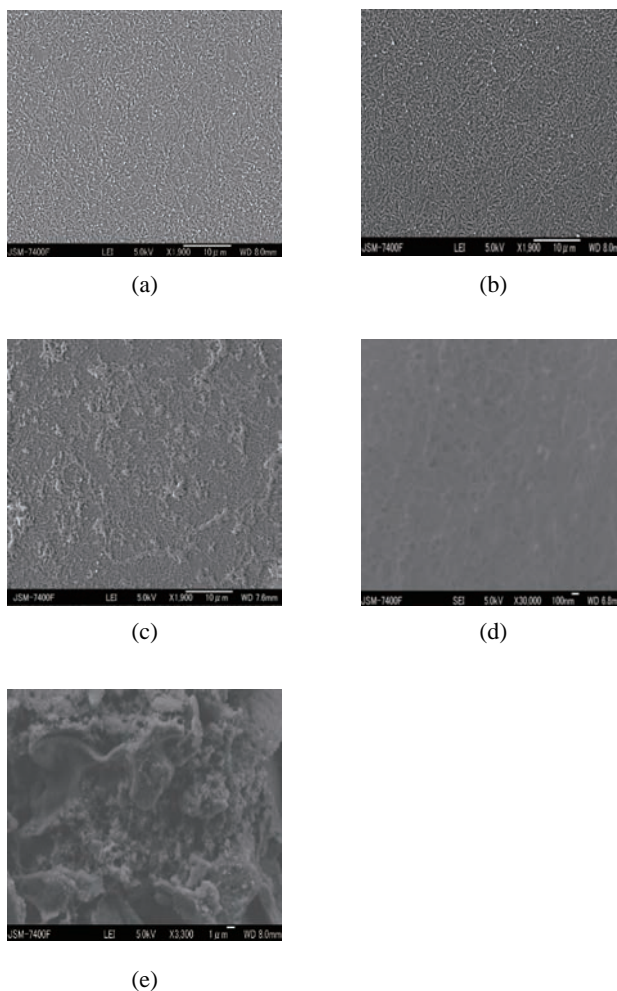
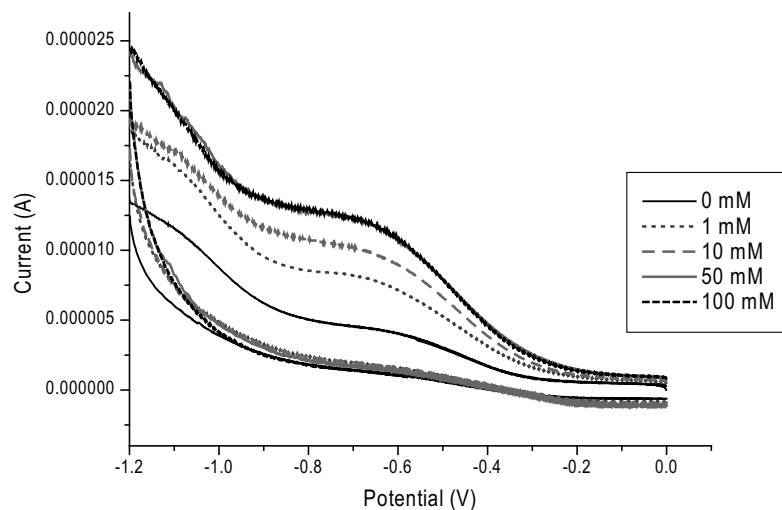


Fig. 2. SEM images of plasma-polymerized CoTPP film on ITO (a) 30 W and (b) 50 W, (c) CoTPP/ITO after immobilizing the enzyme GOx, (d) SWCNTs drop-casted on CoTPP/ITO substrates, (e) CoTPP coating over SWCNT/CoTPP/ITO electrodes.

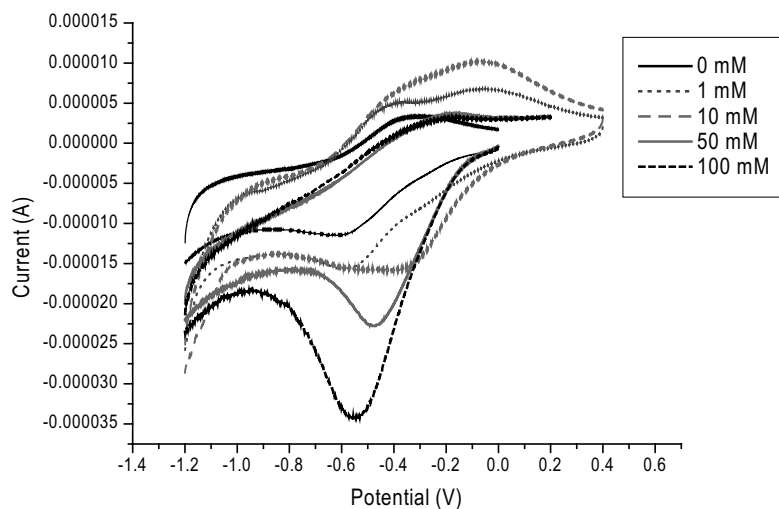
3.2 Sensor characteristics

3.2.1 Devices with plasma-polymerized CoTPP deposition using 50 W power

Figures 3(a) and 3(b) depict the cyclic voltammograms of GOx/CoTPP/ITO and GOx/CoTPP/SWCNT/CoTPP/ITO electrodes in response to glucose. Both devices show



(a)



(b)

Fig. 3. (a) Cyclic voltammograms of GOx/CoTPP/ITO device (plasma power: 50 W) without SWCNTs in 0.1 M PBS in response to glucose. (b) Cyclic voltammograms of GOx/CoTPP/SWCNT/CoTPP/ITO (plasma power: 50 W) device in 0.1 M PBS in response to glucose.

an improvement in sensor response corresponding to an increase in glucose concentration from 0 to 100 mM. The sensor response was largely elevated in electrodes containing SWCNTs compared with that in electrodes without SWCNTs. The GOx-immobilized CoTPP/ITO device shows only a slight variation in sensor response to glucose as the concentration of glucose increases from 0 to 100 mM [Fig. 3(a)]. A prominent increase in sensor current was obtained in the case of the SWCNT-coated CoTPP/ITO device [as the glucose concentration increases from 0 to 100 mM; Fig. 3(b)]. The presence of SWCNTs on plasma-polymerized CoTPP thin film enhances the electron transfer between ITO and GOx resulting in the increased sensor response. The large current response can also be explained by the better catalytic activity of CNTs towards hydrogen peroxide generated during the enzymatic reaction.^(33,35) Thus, the use of SWCNTs with plasma-polymerized thin film of CoTPP enhances the sensitivity of these devices in response to glucose.

Figure 4 shows the calibration curve of the sensor response of GOx/CoTPP/SWCNT/CoTPP/ITO devices to glucose concentration. The linear relationship obviously shows the potential for application of this device as a glucose sensor. It is evident from the graph that the sensor response increases with a corresponding increase in glucose concentration. It exhibits a 234 nA change in current for an increase of 1 mM glucose in solution. This sensor response is very much higher than that of the device without SWCNTs (GOx/CoTPP/ITO), which clearly indicates that our device with SWCNTs is suitable for glucose sensing. The nonlinear relationship between the sensor response and glucose concentration for GOx/CoTPP/ITO devices (without SWCNTs) reveals that the GOx-immobilized CoTPP/ITO device (deposition of CoTPP with a plasma power of 50 W) is not favorable for glucose sensing purposes.

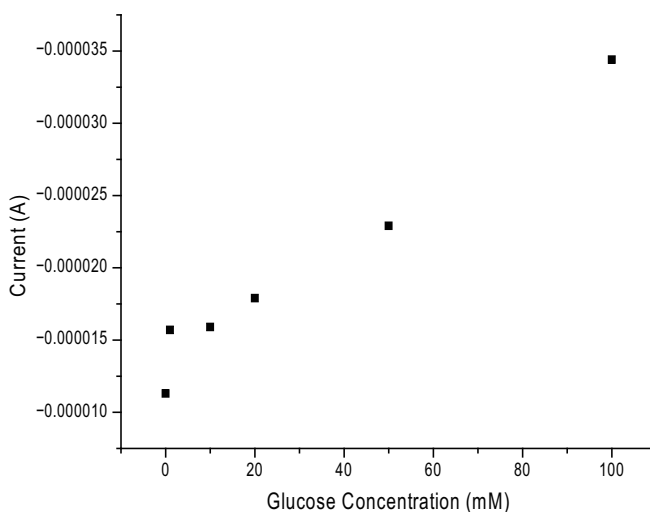


Fig. 4. Calibration curve of GOx/CoTPP/SWCNT/CoTPP/ITO device (plasma power: 50 W) in response to glucose concentration.

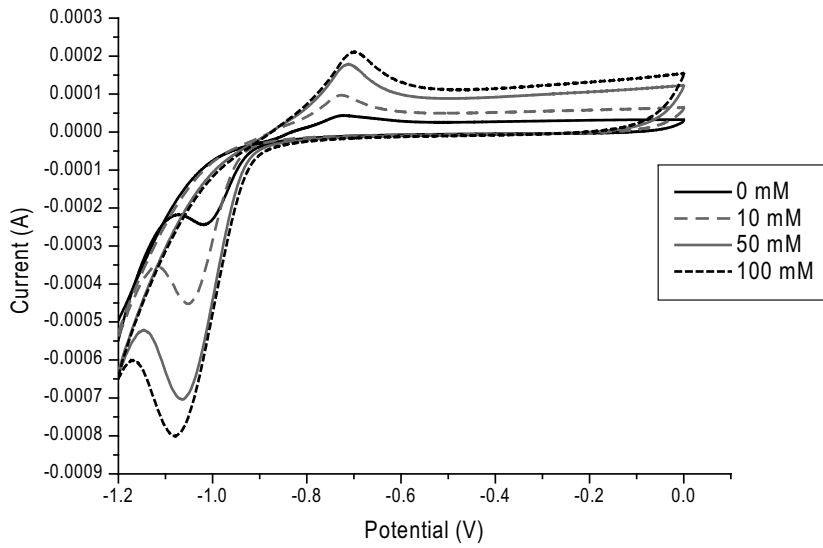
3.2.2 Devices with plasma-polymerized CoTPP deposition using 30 W power

In order to check whether polymer films deposited with low rf powers are suitable for glucose sensing purposes, we carried out cyclic voltammetric experiments with a plasma-polymerized CoTPP film produced at 30 W rf power. The cyclic voltammograms for GOx/CoTPP/ITO and GOx/CoTPP/SWCNT/CoTPP/ITO electrodes in response to glucose are given in Figs. 5(a) and 5(b), respectively. As the concentration of glucose increased from 0 to 100 mM, the sensor response of both devices increased gradually. Both oxidation and reduction peaks were obtained for both devices and the sensor response of devices containing SWCNTs was enhanced compared with that of electrodes without SWCNTs. As both devices (with and without SWCNTs) show different oxidation and reduction peaks for corresponding changes in glucose concentration, both are good candidates for glucose sensing applications.

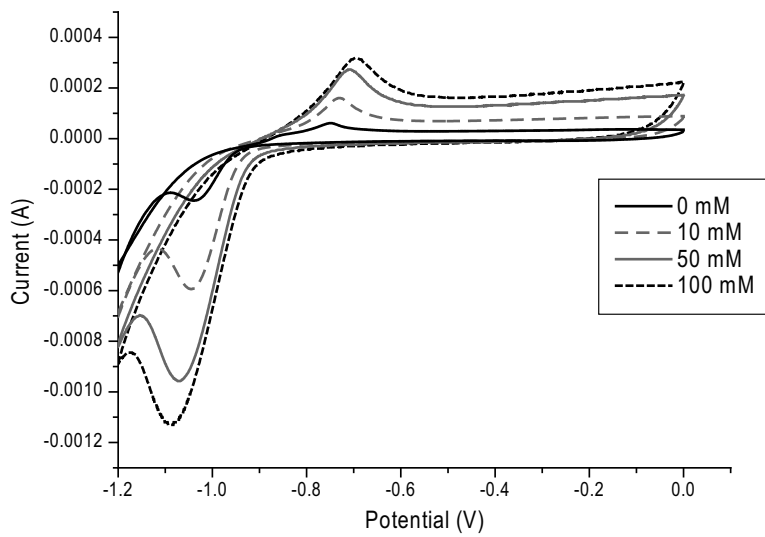
Figure 6 shows the calibration curves of sensor responses of GOx/CoTPP/SWCNT/CoTPP/ITO and GOx/CoTPP/ITO devices to glucose concentration. We found that there is a linear relationship between glucose concentration and sensor response for both devices. It is calculated from Fig. 6(a) that there is 1.75 μA change in current for an increase of 1 mM glucose in solution for devices without SWCNTs. The SWCNT-deposited devices [Fig. 6 (b)] show a 3.67 μA change in current (about twice the current obtained for devices without SWCNTs) corresponding to a 1 mM increase in glucose concentration in solution. This again confirms that the use of SWCNTs along with plasma-polymerized CoTPP thin film improves and enhances the sensitivity of these devices in response to glucose.

The reproducibility of GOx/CoTPP/SWCNT/CoTPP/ITO and GOx/CoTPP/ITO devices produced at 30 and 50 W was estimated by conducting CV experiments using five more devices. Our devices show satisfactory repeatability of measurements to different concentrations of glucose in 0.1 M PBS. This provides evidence of the reliability of our devices.

The sensitivities of all these devices towards glucose with different plasma powers (30 and 50 W) of polymer deposition over ITO were also compared. When we reduced the plasma power to 10 and 20 W for the thin-film deposition of CoTPP over ITO substrates, we noticed that the adhesion strength of the obtained protective film was very low. However, the films produced at 30 and 50 W show good adhesion to ITO substrates. Since the adhesive power to substrates is a crucial factor in designing sensor devices, we selected high plasma powers (30 and 50 W) to coat protective films of CoTPP over evaporated CoTPP-ITO substrates. The devices coated with CoTPP with 30 W plasma power show higher sensitivity than the devices coated with polymer with 50 W power. The reduction of the sensitivity of devices with a protective layer of plasma-polymerized CoTPP thin films produced at 50 W may be due to the highly altered and damaged states of thin films. Thus, we can conclude that polymer thin films produced at a moderate plasma power of 30 W are highly suitable for use as a protective layer in glucose sensing applications compared with thin films produced at a high plasma power of 50 W. However, the use of SWCNTs enhances the electrochemical communication between the enzyme and ITO substrates through the CoTPP polymer thin film, thereby improving the sensitivity of both devices.



(a)



(b)

Fig. 5. (a) Cyclic voltammograms of GOx/CoTPP/ITO (plasma power: 30 W) in 0.1 M PBS (pH 7.0) in response to glucose. (b) Cyclic voltammograms of GOx/CoTPP/SWCNT/CoTPP/ITO devices (plasma power: 30 W) in 0.1 M PBS (pH 7.0) in response to glucose.

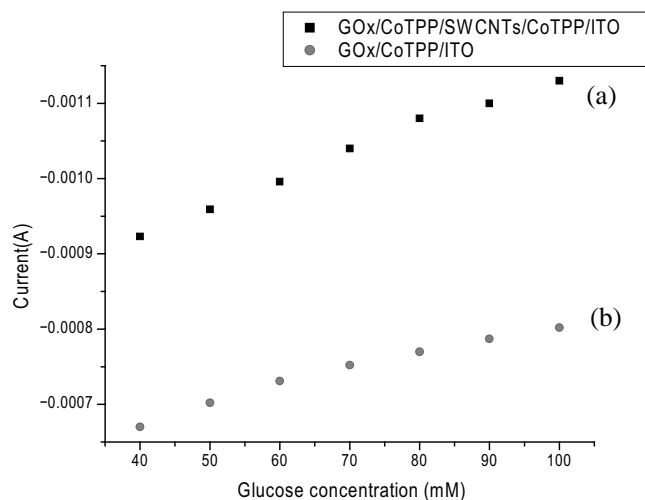


Fig. 6. Calibration curves of (a) GOx/CoTPP/SWCNT/CoTPP/ITO device (30 W) and (b) GOx/CoTPP/ITO device (30 W) in response to glucose concentration.

4. Conclusions

We have described a very simple and easy method of developing an electrochemical glucose biosensor based on SWCNTs. A plasma-polymerized thin film of CoTPP coated on ITO substrates serves as an interfacial design between GOx and ITO electrodes and also hastens the electrochemical communication between the enzyme and ITO. The presence of SWCNTs along with the plasma-polymerized CoTPP polymer enhances this electron transfer, which results in the higher sensing response of devices. Our device based on SWCNTs (GOx/SWCNT/CoTPP/ITO) shows higher sensitivity than devices without SWCNTs (GOx/CoTPP/ITO). The plasma power also affects the sensitivity and we found that the sensitivity increases with decreasing plasma power. Our results suggest that SWCNTs and plasma-polymerized CoTPP thin-film-based devices are promising candidates for glucose sensing.

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