

Cobalt Tetraphenylporphine (CoTPP) Film as Glucose and Hydrogen Peroxide Sensors after Immobilizing Glucose Oxidase (GOx)

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We developed a cobalt tetraphenylporphine (CoTPP) thin film by vacuum evaporation and plasma polymerization and checked its utility as a glucose and hydrogen peroxide (H_2O_2) sensor. The sensing ability was realized by immobilizing glucose oxidase (GOx) enzyme on the surface of the plasma-polymerized CoTPP film. The plasma-polymerized film acts as a protective layer to the vacuum-evaporated CoTPP film. Cyclic voltammetry has been used to characterize the sensing behavior of the combination of indium tin oxide (ITO-acting as an electrode), CoTPP film (both vacuum evaporated and plasma polymerized) and the GOx layer. Our results suggest that we can use the ITO, CoTPP and GOx combination as a very good glucose and H_2O_2 sensor.

1. Introduction

Nowadays, sensors are giving rise to a great deal of interest due to their applications in various fields such as biology, medicine, safety and environmental protection. The second half of the 20th century witnessed the development of sensing devices and their commercial applications. In the initial days of the development of sensors, we relied mainly on inorganic materials. However, later, organic polymers also captured our attention because they offer many advantages for sensor technologies, such as the production of relatively low-cost materials, simple fabrication techniques (no need for any clean room, etc.), the possibility of deposition on various types of substrates, a wide choice of molecular structures, and the prospect of generating side chains and charged or neutral particles.^(1,2)

When considering sensors based on polymers, it is worth noting that plasma-polymerized polymer sensors also play an important role in this field, along with other polymer sensors with and without immobilized enzymes on their surfaces.^(2–9) Since the plasma-

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polymerized polymer is highly cross-linked and pinhole free, the enzyme, having a large molecular size, remains immobilized on the surface.⁽³⁾ Plasma polymerization allows the deposition of very thin and stable polymer films that are insoluble even in organic solvents and strongly adhering to the substrate materials. Such films can be homogeneously deposited over a relatively large area. Janasek et al. used a plasma-polymerized polymer layer as a protective layer in their hydrogen peroxide (H_2O_2) sensor because of the above-mentioned characteristic features.⁽⁷⁾

In recent years, several authors have put forward the idea of using porphyrins and phthalocyanines as sensing elements.^(10–14) The use of porphyrins and metalloporphyrins has been proposed for gas sensing, mainly oxygen, NO_2 , NH_3 , H_2S , CO_2 , HCl , alcohols and amines.⁽¹⁰⁾ Recently, cobalt phthalocyanines have been used to detect glucose concentration using glucose oxidase (GOx—an enzyme commonly used in glucose-sensing devices)⁽¹⁵⁾ because phthalocyanine acts as an electrocatalyst for the oxidation of the H_2O_2 produced by the oxidase reactions. It is reported that metal porphyrins have chemical structures and functionalities similar to those of metal phthalocyanines, and are promising as organic electronic materials having higher functionalities than phthalocyanines and their derivatives.⁽¹⁶⁾ Cobalt tetraphenylporphine (CoTPP) has been used in ChemFET sensors as the sensitive layer for gas sensing⁽¹⁷⁾ and for detecting amines.⁽¹⁰⁾ It is reported that CoTPP has been used as a catalyst for the electroreduction of O_2 .⁽¹⁸⁾ GOx is an enzyme that converts glucose into gluconic acid and H_2O_2 , while at the same time reducing dioxygen (O_2) to water. Because of this property, many authors have reported glucose and H_2O_2 sensors using GOx.^(15,19–23)

Considering the above facts, we have tried to exploit the advantage of the catalytic activity of CoTPP to construct a glucose and H_2O_2 sensor by developing CoTPP thin films. However, we found that vacuum-deposited CoTPP shows similar characteristics to those of its monomer, i.e., solubility in water and very weak adhesion to the substrate, as well as only meager hardness, which are not favorable characteristics for achieving our aim. Here, the characteristic features of plasma-polymerized polymers, such as hardness, toughness and insolubility even in organic solvents,⁽²⁴⁾ allow such films to play important and crucial roles as protective layers of vacuum-deposited CoTPP film. Therefore, we have developed a thin plasma-polymerized polymer film as a protective layer on the evaporated CoTPP film. Consequently we gained the advantage of immobilizing enzymes on the surface of the plasma-polymerized polymers.⁽³⁾ Here, in the present paper, we report the role of GOx-immobilized plasma-polymerized CoTPP films deposited on CoTPP vacuum-evaporated film as a glucose and H_2O_2 sensor.

2. Experimental Setup

The preparation procedure of CoTPP and some characterizations of the plasma-polymerized CoTPP films can be found in one of our previous publications.⁽²⁵⁾ Figure 1 shows the chemical structure of CoTPP. Figure 2 depicts the apparatus arrangement for vacuum deposition and plasma polymerization of CoTPP. The apparatus consisted of a Pyrex glass bell jar, a pair of parallel disk electrodes (70 mm in diameter; the lower electrode connected to a heater and the upper one connected to a radio-frequency (rf)

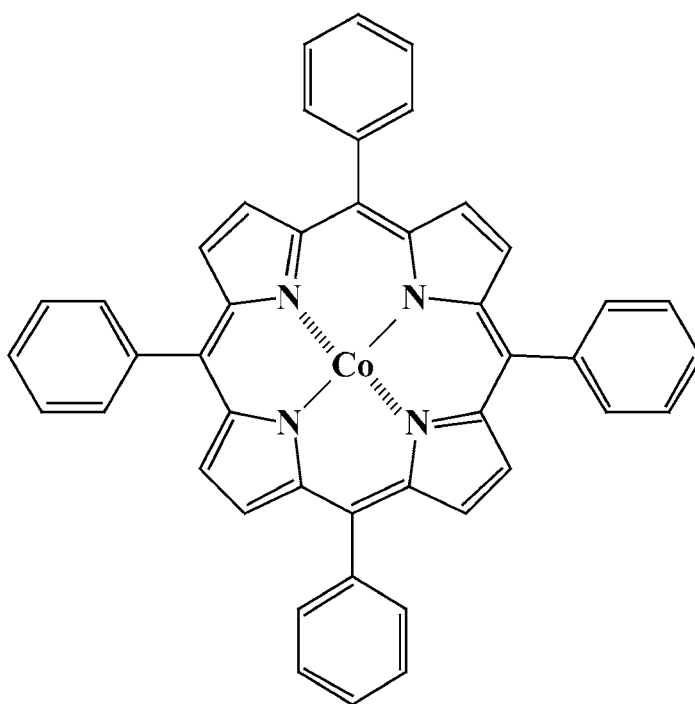


Fig. 1. Chemical structure of CoTPP.

generator connected to an RFG-200 radio-frequency generator operated at 13.56 MHz, through an impedance-matching circuit (Samco International, model BP-1). We deposited the thin films of CoTPP on substrates made of ITO, quartz plate and slide glass by placing these substrates on the lower side of the upper electrode located 25 mm above the lower electrode at the center of the reactor.

For the deposition of CoTPP we placed CoTPP powder on the lower electrode and heated the material to 380°C (after the chamber evacuated to 5 Pa) with the shutter closed. (In the plasma polymerization chamber, the shutter was introduced between the upper and lower electrodes which was different from our previous setup⁽²⁵⁾ to prevent the excess deposition of CoTPP material during heating from room temperature to 380°C). Upon reaching 380°C, the evaporated CoTPP was deposited on the substrates between the temperatures of 380 and 390°C by opening the shutter (This deposition of CoTPP thin film is the vacuum-evaporated layer). After reaching 390°C, we employed plasma discharge for 60 s to coat a protective plasma-polymerized film onto the evaporated film at different plasma powers. Plasma-polymerized CoTPP thin films were prepared by the rf discharge of argon gas at a pressure of 10 Pa, and a flow rate of 5 mL/min. An rf power in the range from 10 W to 100 W was introduced into the reactor through a matching box.

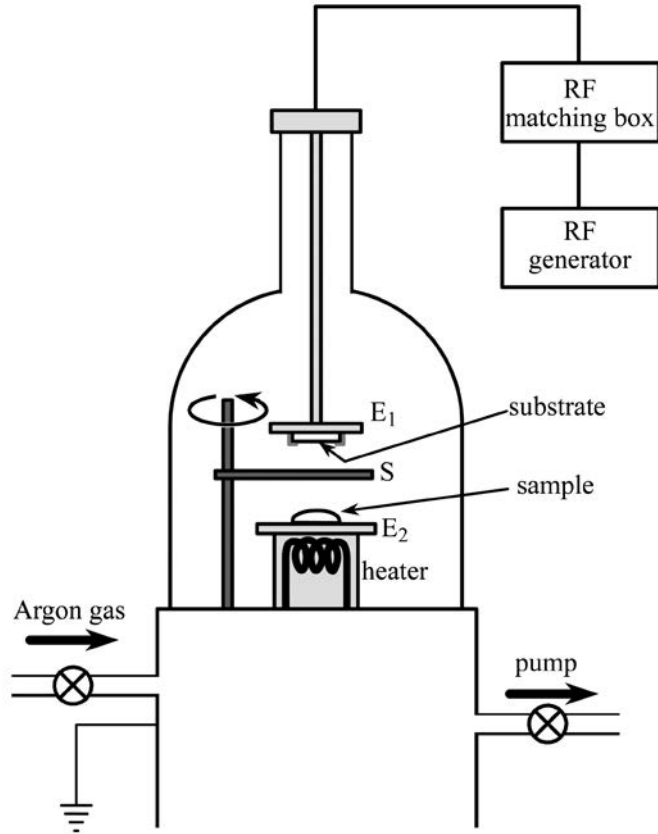


Fig. 2. Thin film deposition chamber for vacuum-evaporated deposition and plasma-polymerization. E₁ and E₂ are the upper and lower electrodes, respectively, and S denotes the shutter.

The adhesive force of the plasma-polymerized films to the glass substrates was estimated by the cross-cut test, in which adhesive tape is used to peel off the thin film cross-cut into 100 squares (10×10; 1×1 mm each) from the glass plate. The adhesive strength is expressed by an index from '0' to '10', according to the extent of retention of the squares after peeling off the tape. The index '0' means complete detachment of the cross-cut squares and the index '10' means no detachment of the cross-cut squares. The hardness of plasma-polymerized CoTPP thin films was measured by the scratch test with pencils of different hardnesses from HB to 9H (JIS; K5400-1900). UV-VIS spectra of the thin films on quartz plates were obtained in the transmission mode using a spectrometer (Shimadzu,

UV-2100S) in the wavelength range of 350 nm to 700 nm. We performed cyclic voltammetry studies for characterizing the glucose and H₂O₂-sensing behaviors of the CoTPP films fabricated onto an area of 1 cm².

2.1 Construction of sensor device

Figure 3 depicts the schematic diagram of our sensing device. To fabricate our sensor device, we coated CoTPP films (area 1 cm²; both evaporated and plasma polymerized) on ITO substrates using a proper mask. To introduce the GOx enzyme (obtained from *aspergillus niger*, lyophilized 8 U/mg for biochemistry, Merck) onto the surface of the plasma-polymerized protective film, the substrate with the deposited film was immersed in GOx solution (250 mg of GOx in 5 ml distilled water) at 30°C for 30 min. For cyclic voltammetry studies, we used a working electrode of ITO/ CoTPP thin films (both vacuum evaporated and plasma polymerized)/ GOx in 0.1 M phosphate buffer solution (pH 7.0), a counterelectrode of Pt, and a reference electrode of Ag/AgCl.

3. Results and Discussion

We deposited plasma-polymerized CoTPP film as a protective layer (prepared under various plasma powers from 10 W to 100 W) onto the evaporated CoTPP films. Since the adhesive force and hardness of this film are crucial in the development of the sensor devices, we have characterized these properties of the films. Table 1 shows the results of the cross-cut test for the plasma-polymerized CoTPP films deposited on glass substrates at different rf plasma powers. The results are similar to those obtained previously.⁽²⁵⁾ These results suggest that the polymer films produced at relatively low plasma powers (10 and 20 W) could be peeled off very easily from the glass substrates. However, the adhesive force of the plasma-polymerized thin films on the glass substrates was increased with increasing rf power. The plasma-polymerized CoTPP thin films obtained at rf powers of 30 W and higher adhered to glass substrates very strongly. Regarding the adhesive force, almost the

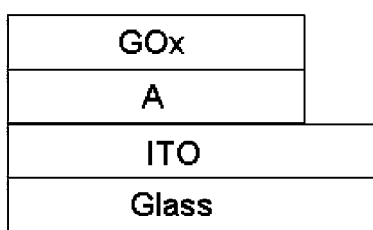


Fig. 3. Schematic diagram of sensor device. 'A' denotes vacuum-evaporated CoTPP film with protective plasma-polymerized film.

Table 1
Adhesive force and hardness of vacuum-deposited and plasma-polymerized CoTPP thin films.

Characterization method	Vacuum deposition	Plasma polymerization				
		10 W	20 W	30 W	50 W	100 W
Adhesive force ^a	0	2	4	10	10	10
Hardness ^b	HB	H	6H	7H	9H	9H

^a The adhesive force of thin film to glass was estimated by the cross-cut test. (0: complete detachment of cross-cut squares. 10: no detachment of cross-cut squares).

^b The hardness of thin film was measured by the scratch test with pencils of various hardnesses from HB to 9 H.

same results were obtained for ITO-coated substrates. Almost the same trend, i.e., an increase in the hardness with increasing rf power, was also observed for plasma-polymerized films.

In our previous UV-VIS characterizations (Fig. 4) of CoTPP films,⁽²⁵⁾ we found that the film produced by vacuum deposition at rf power of 10 W almost completely retains the chemical structure of CoTPP. However, the adhesion strength of this film to the substrate was found to be very low. Films produced at 30 W to 100 W showed very good adhesion, and films produced at 30 W retained the chemical structure, to a certain extent, compared with the films produced at higher powers.⁽²⁵⁾ Since adhesion, hardness and retention of the chemical structure of the CoTPP films are crucial factors when considering sensor preparation, as a compromise we used the films produced at the rf power of 30 W as the protective coating on the evaporated CoTPP films.

Since CoTPP shows catalytic behavior, we analyzed the sensing behavior of the ITO/CoTPP combination for different glucose concentrations using cyclic voltammograms. Figure 5 shows reduction peaks obtained using the ITO/CoTPP combination for different glucose concentrations. Even though we observed some reduction peaks, we could not differentiate between the concentrations of glucose using this system because this system yielded almost the same reduction peaks regardless of the glucose concentration. This clearly means that CoTPP alone cannot act as the glucose sensor.

3.1 Sensor characterization

Figure 6 shows the cyclic voltammograms for ITO/CoTPP-evaporated film protected by plasma-polymerized film (30 W)/GOx in 0.1 M phosphate buffer solution (pH 7.0) for different H₂O₂ concentrations. We obtained different reduction peaks for each concentration of H₂O₂, which enabled us to differentiate between the concentrations. Figure 7 represents the calibration curve of the sensor response to H₂O₂ concentrations. The linear relationship obtained in this graph clearly indicates the very good sensor response of this

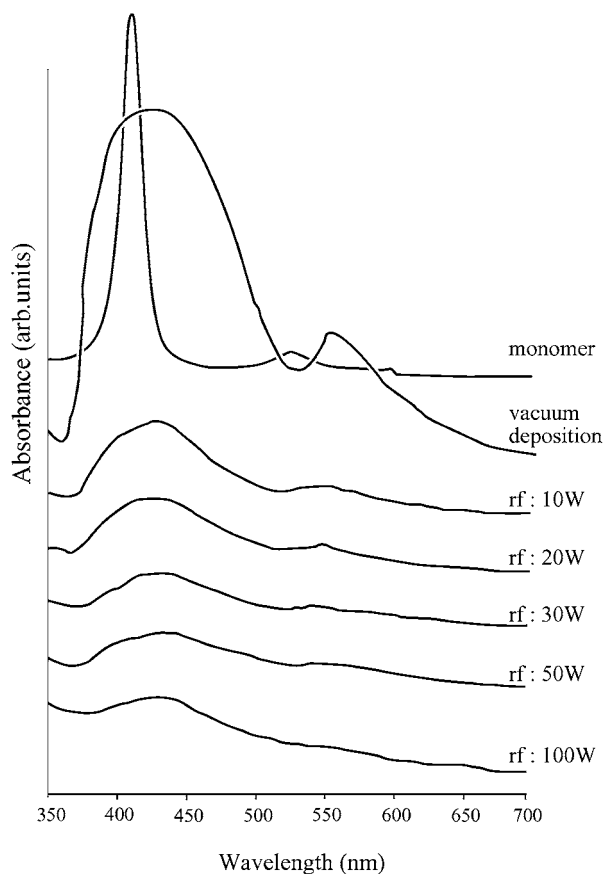


Fig. 4. UV-VIS spectra of the CoTPP monomer solution in CH_2Cl_2 , and the vacuum-deposited and plasma-polymerized CoTPP thin films.

system. From this figure, we can obtain the information that for each increment of 1 mM of H_2O_2 , there is a change of about $3.75 \mu\text{A}$ in the sensor current.

Figure 8 shows the cyclic voltammograms of ITO/ CoTPP-evaporated film protected by the plasma-polymerized film (30 W)/GOx combination in 0.1 M phosphate buffer solution for different glucose concentrations. In this case, we also obtained different reduction peaks for different glucose concentrations, which enabled us to differentiate between the glucose concentrations. Using the different reduction peaks obtained for different glucose concentrations employing the sensor device with the plasma-polymerized films produced at 30 W, we plotted a semilog graph of sensor response to glucose concentration, as shown in Fig. 9. The linear relationship of this graph suggests that this combination is promising for developing a sensor device. From this graph, we obtained an increment of $34.3 \mu\text{A}$ for an increase of 1 mM of glucose in the solution as the sensor response, which is promising for developing a sensor device.

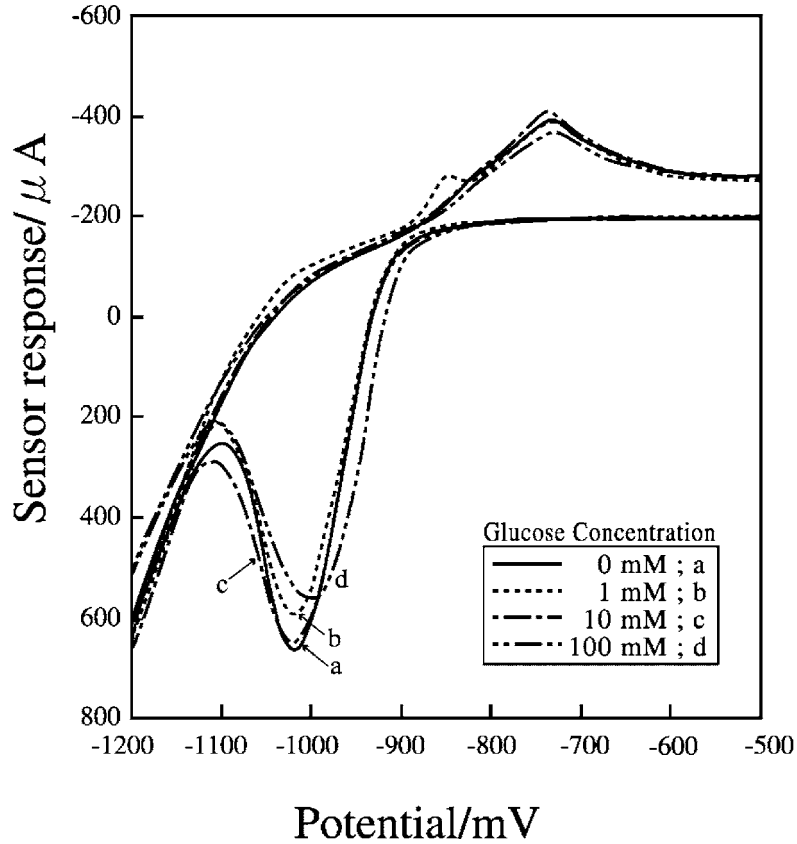


Fig. 5. Cyclic voltammograms for ITO/plasma-polymerized CoTPP thin film (rf 30 W) in 0.1 M phosphate buffer solution (pH 7.0) in response to glucose.

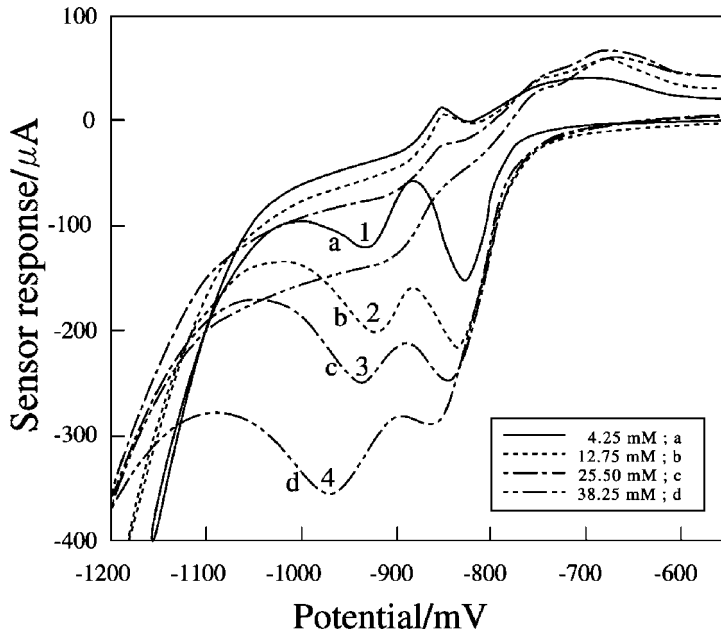


Fig. 6. Cyclic voltammograms for ITO/CoTPP-evaporated film protected by plasma-polymerized film (30 W)/GOx in 0.1 M phosphate buffer solution (pH 7.0) in response to H₂O₂. (Numbers 1, 2, 3 and 4 represent the reduction peaks.)

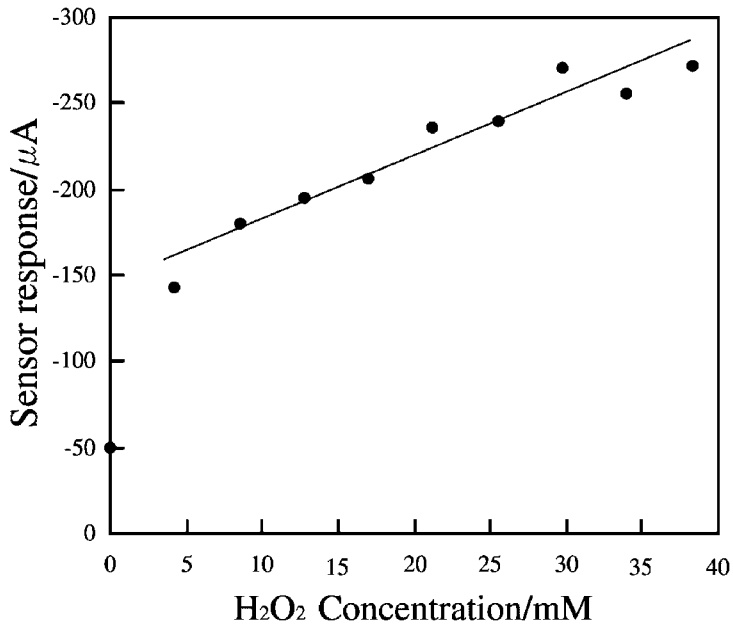


Fig. 7. Calibration curve for the sensor response to H₂O₂ concentration.

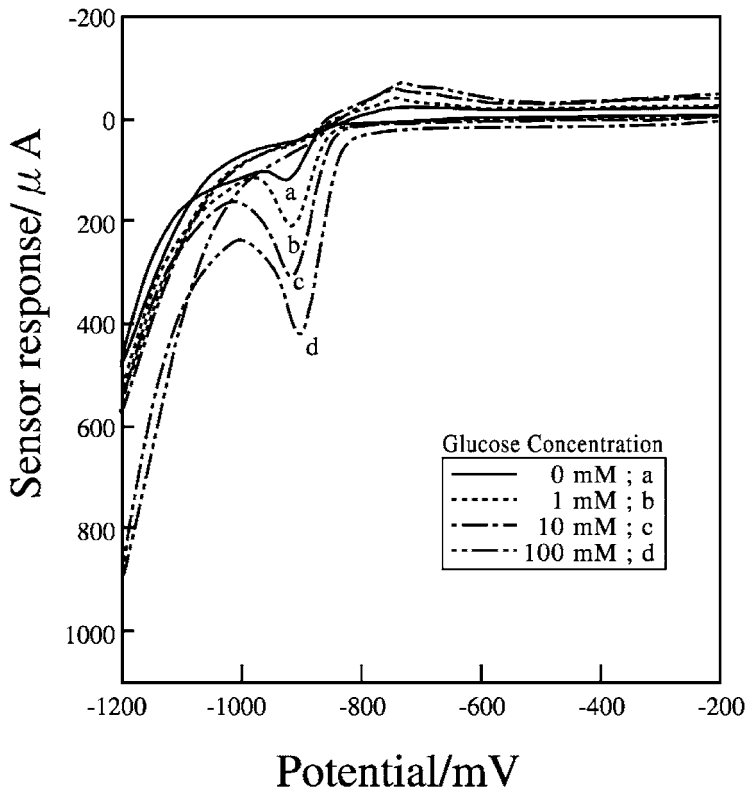


Fig. 8. Cyclic voltammograms for ITO/ CoTPP-evaporated film protected by plasma-polymerized film (30 W)/GOx in 0.1 M phosphate buffer solution (pH 7.0) in response to glucose.

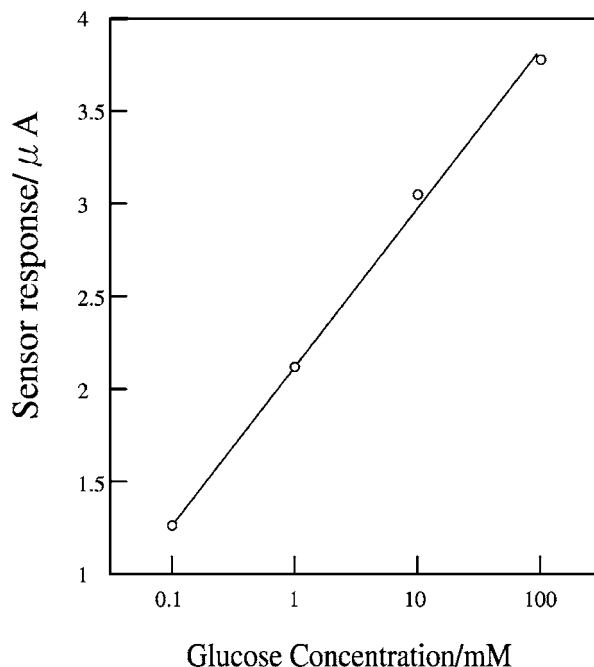


Fig. 9. Calibration curve for sensor response to glucose concentration represented on semilog graph.

For comparison and confirmation that the protective plasma-polymerized polymer films produced at higher rf powers are not useful for the desired purpose because of their highly altered chemical structure (as evidenced from the UV-VIS studies), we used the same sensor setup but changed the powers of plasma polymerization to 50 and 100 W. The sensor responses of the resultant films used as protective layers are shown in Figs. 10 and 11, respectively. We found that using the protective layers of plasma-polymerized films produced at higher plasma powers does not provide good results. We can see that the reduction peaks do not increase linearly, which is unfavorable for sensor equipment. This may be due to the highly cross-linked and damaged state of the polymer films produced at these higher powers.

To check how the CoTPP plasma-polymerized film alone without using the CoTPP evaporated films responds to the glucose concentration, we deposited the plasma-polymerized film of 30 W rf power after opening the shutter of the plasma polymerization chamber for 60 s upon reaching the heating temperature of 390°C. After depositing the film, we treated the sample in the same manner as described above to adhere the GOx onto the surface. However we obtained cyclic voltammetry graphs similar to the ones shown in Fig. 11 for 100 W. This clearly shows that evaporated CoTPP films, with little damage during deposition are need to achieve good catalytic activity.

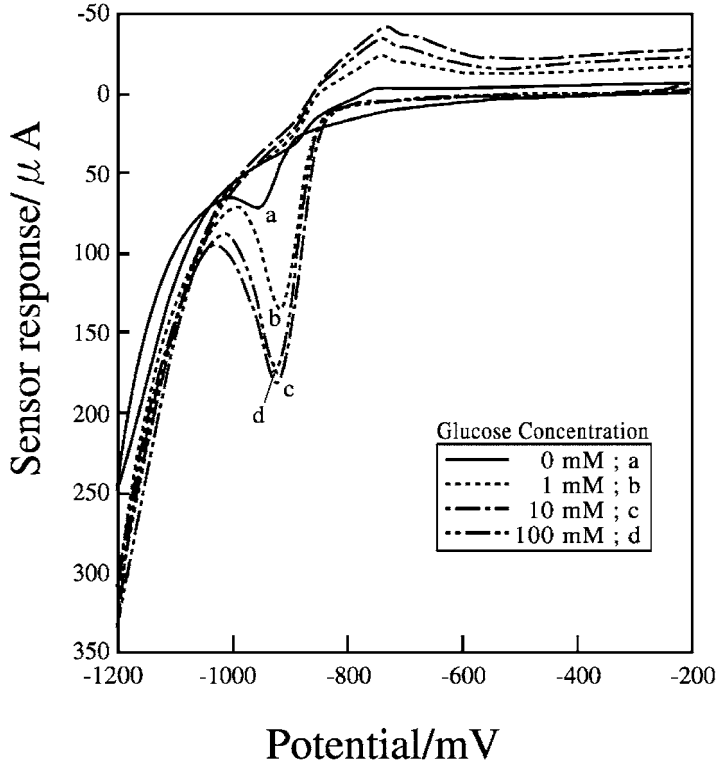


Fig. 10. Cyclic voltammograms for ITO/ CoTPP-evaporated film protected by plasma-polymerized film (50 W)/GOx in 0.1M phosphate buffer solution (pH 7.0) in response to glucose.

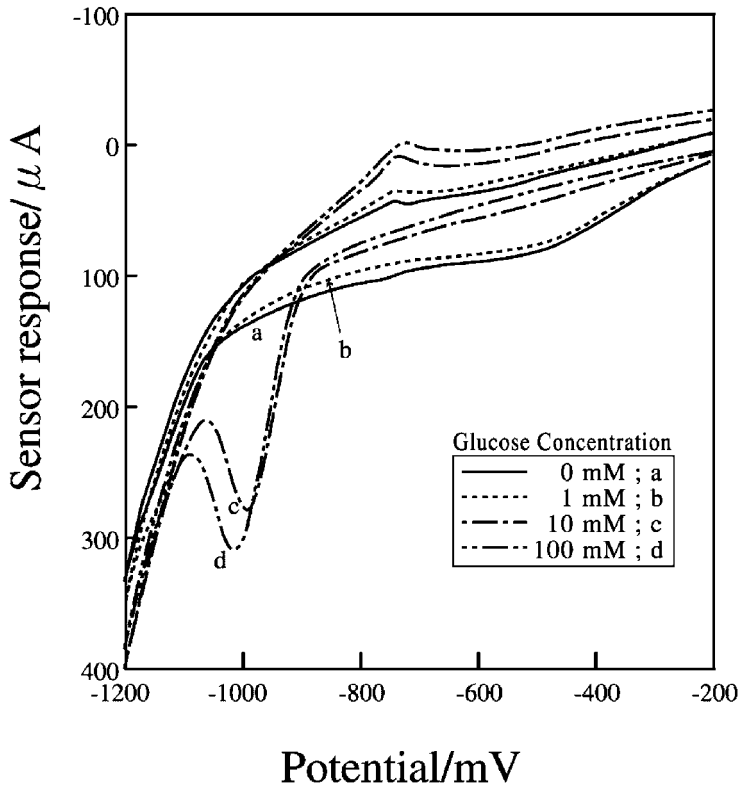


Fig. 11. Cyclic voltammograms for ITO/ CoTPP-evaporated film protected by plasma-polymerized film (100 W)/GOx in 0.1 M phosphate buffer solution (pH 7.0) in response to glucose.

We found that our system shows good repeatability even after keeping the system for 1 week and after 10 cycles of repeated operations. This result clearly indicates the reliability of our system. When not in use, the sensors were stored in PBS solution in a refrigerator.

4. Conclusion

We found that GOx-immobilized CoTPP films (vacuum-evaporated/ plasma-polymerized films) are good candidates for detecting glucose as well as H₂O₂. We demonstrated that, without GOx or vacuum-evaporated CoTPP film, the system will not perform in the desired manner. Even though the films grown at low plasma power show good chemical structure and retainability, considering their very low adhesion strength to the substrates, it is proposed that films produced at moderate plasma power (for example, 30 W) are better for use as the protective layer in sensor applications. Furthermore we found that due to the high damage to the chemical structure of plasma-polymerized films produced at 50 and 100 W, they cannot be used as protective layers.

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