Glucose oxidase immobilization on different modified surfaces of platinum nanowire for application in glucose detection

Thi Thanh Tuyen Le, Phu Duy Tran, Xuan Tung Pham, Duy Hien Tong and Mau Chien Dang

Laboratory for Nanotechnology, Vietnam National University–Ho Chi Minh City Community 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
E-mail: lttuyen@vnuhcm.edu.vn

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Abstract
In this work, the surface of platinum (Pt) nanowires was modified by using several chemicals, including a compound of gelatin gel with SiO$_2$, polyvinyl alcohol (PVA) with Prussian blue (PB) mediator and cysteamine self-assembled monolayers (SAM). Then, glucose oxidase (GOD) enzyme was immobilized on the modified surfaces of Pt nanowire electrodes by using techniques of electrochemical adsorption and chemical binding. The GOD immobilized Pt nanowires were used for application in glucose detection by performing a cyclic voltammetry measurement. The detection results showed that GOD was immobilized on all of the tested surfaces and the highest glucose detection sensitivity of 60 $\mu$M was obtained when the Pt nanowires were modified by PVA with PB mediator. Moreover, the sensors showed very high current response when the Pt nanowires were modified with the cysteamine SAM. The stability and catalyst activity of GOD are also reported here. For instance, the catalyst activity of GOD retained about 60% of its initial value after it was stored at 4 $^\circ$C in a 100 mM PBS buffer solution with a pH of 7.2 for a period of 30 days.

Keywords: glucose oxidase, immobilization, biosensor, nanowire

Classification numbers: 4.08, 6.09

1. Introduction
Biosensors, particularly enzyme-based amperometric sensors, have been studied extensively because of their scientific significance and commercial potential in both academic and applied fields [1–6]. Recently, nano-scale devices based on nanowires have been given special attention due to their broad applications and advantages in different sectors, ranging from electronics to optics, gas sensing and especially biomedical diagnostics. Among the developed devices, glucose electrochemical biosensors based on the enzymatic oxidation of glucose oxidase (GOD) onto platinum nanowires have generated considerable interest due to their advantages of high sensitivity and selectivity, quick response time, etc.

In these sensors, the immobilized GOD enzyme catalyzes the oxidation of glucose to gluconolactone in the presence of oxygen, while coenzyme flavinadenindinucleotide (FAD) is reduced to FADH$_2$. In the natural enzymatic reaction, molecular oxygen functions as an electron acceptor for FADH$_2$ and reoxidizes FADH$_2$ to FAD, whereas O$_2$ is reduced to H$_2$O$_2$. The H$_2$O$_2$ is then detected by amperometric measurement, allowing the determination of the corresponding glucose concentration of the solution,

$$\text{glucose} + \text{O}_2 \rightarrow \text{gluconolactone} + \text{H}_2\text{O}_2,$$

(1)

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-.$$  

(2)

Apparently, in the above detection sequence, the interaction between immobilized enzyme molecules and the
platinum electrode surface has a critical influence on the final sensor signal. In this work, we immobilized GOD onto platinum nanowires by using different techniques to investigate three generations of glucose sensor. In the first generation, enzymes were immobilized via membrane silica–gel (SiO$_2$ + gelatin). This membrane creates a flexible matrix, negligible swelling in aqueous solution and thermal stability on the electrode [1]. In the second generation, GODs were immobilized through a polyvinyl alcohol (PVA) layer and a Prussian blue (PB) mediator. In the last generation, GOD immobilization influence was also studied for the self-assembled monolayers (SAMs) of cysteamine onto the platinum surface [3]. In addition, the performance of the glucose biosensors, including the response time, enzymatic sensitivity and device durability, are reported.

2. Experimental

2.1. Reagents and apparatus

D-glucose and glucose oxidase (GOx, EC 1.1.3.4, 172 000 units g$^{-1}$ from Aspergillus niger) were purchased from Sigma Aldrich. Gelatin (Merck) solution was dissolved in 0.05 M acetate buffer pH 5.5 (CH$_3$COOH, CH$_3$COONa) and stirred for 1 h at 70 °C. 25 wt% glutaraldehyde solution and tetraethylorthosilicat (TEOS) were purchased from Merck. SiO$_2$ solution was prepared by mixing 0.2 ml TEOS with 20 ml Ethanol 100%, 0.3 ml NH$_4$OH, 0.3 ml H$_2$O and 1 ml HCl in a glass vial. Then the homogeneous solution was obtained by stirring the solution at 80 °C for 7 h. Polyvinylalcohol (PVA), cysteamine and aminopropyltriethoxylane were obtained from Sigma, while potassiumferricyanide (K$_3$Fe(CN)$_6$) and ferricchloride (FeCl$_3$) were obtained from Aldrich. A 0.05 M phosphate buffer (PBS) solution was prepared using Na$_2$HPO$_4$ and KH$_2$PO$_4$. All solutions were filtered through a syringe cellulose acetate (0.22 µm) before use. Double distilled deionized water was used throughout the experiment.

Cyclic amperometric measurements were carried out by an AUTOLAB PGSTAT12/30/302 (Netherlands), and all experiments were carried out with a conventional three-electrode system in a 15 ml electrochemical cell. The working electrode was an array of 20 parallel Pt nanowires, which was fabricated by a deposition and etching under angle method [4, 5, 10]. Each fabricated nanowire is 50 nm in diameter and 20 µm in length. A platinum rod was used as the counter electrode. All of the potentials quoted here were relative to an Ag/AgCl reference electrode and were measured at room temperature.

2.2. Preparation of enzyme electrode on different modified surface of Pt nanowire

Prior to the modification process, a Pt nanowire electrode was cleaned by using oxygen plasma (power of 250 W, 300 s, oxygen flow of 50 sccm). Then it was electrochemically scanned repeatedly until the voltammogram characteristic was obtained. In the first generation of glucose sensor, the cleaned electrode was immersed into the compound of 1 ml gelatin-SiO$_2$ (3:1 v/v mixture of concentrated gelatin, SiO$_2$ stirred in 2 h) and 0.5 ml GOD (5 mg ml$^{-1}$ of acetate buffer, pH 5.5) solution. Afterwards, the electrode was dried at 4 °C and washed with DI water before being used for glucose detection. In the next experiment, the electrode was reduced by scanning it in 0.001 M H$_2$SO$_4$. Then it was soaked into an ethanol solution containing cysteamine 0.25 M at 4 °C for 12 h. Afterwards, this electrode was immersed into glutaraldehyde (GAD) solution (5 mg ml$^{-1}$ of PBS buffer) for 2 h. Finally, the modified electrode was soaked in GOD solution to bind the free enzyme from the solution onto the platinum surface.

Following the study of enzyme immobilization, PB film was electrodeposited onto the Pt nanowire surface by scanning the solution of 30 mM K$_3$Fe(CN)$_6$, 40 mM FeCl$_3$ and 1 M KCl:1 M HCl solution. The potential was scanned between −0.2 V to 0.8 V with 50 mV s$^{-1}$ in scan rate. In order to firm the PB mediator, we scanned it in 1 M KCl between −0.2 and 0.8 V. Then the modified electrode was immersed successively in PVA (5 mg ml$^{-1}$) solution and aminopropyltriethoxylane 90% for 30 mins and GOD for 3 h. In these experiments, the electrode was dried before dipping into each solution. All enzyme electrodes were kept at 4 °C until use.

2.3. Experimental conditions

Amperometric experiments were carried out in an electrochemical cell containing 15 ml of 0.05 M phosphate buffer. A holding potential of +0.6 V versus Ag/AgCl was applied on the working electrode. The sample containing glucose was added directly into the electrochemical cell, diluted with PBS buffer to the desired concentration.

3. Results and discussion

3.1. Electrochemical characterization of Pt nanowire

Cyclic voltammograms (CVs) were performed in glucose solution in PBS buffer and a variety of glucose concentrations in water to investigate the influence of electrolyte solution on the platinum electrode prior to the immobilization process. We found that the current response of the electrode did not appropriately change when increasing the concentration of the PBS at 0.2–0.8 V. In contrast, when the concentration of glucose in water increased, then all peak currents decreased immediately (figure 1). That phenomenon proves that all of
Figure 2. CV curves of different concentrations of glucose measured by (A) GOD-gelatin/SiO2-Pt electrode, from down to up 0, 2, 4, 6, 8 and 16 mM; (B) GOD-PVA/PB-Pt electrode, from down to up 0, 2, 4, 8 and 12 mM; (C) GOD-cysteamine-Pt electrode, from down to up 0, 2, 4, 6, 8 and 10 mM.

3.2. Effect of pH on enzyme electrode

The influence of pH buffer solution on glucose detection has been studied by several authors [7–10]. Investigation of the effect of pH value on the performance of the glucose sensor is very important because the activity of immobilized GOD is pH dependent [8]. In our work, the pH dependence of a modified electrode by PVA compound and PB mediator was evaluated over the pH range from 5.6 to 8.4. When the pH of the buffer was very low or very high, the GOD electrode exhibited low current response to glucose. An optimum response current was observed at a pH value of 7.2.

3.3. Cyclic voltammograms of enzyme electrodes

The response current of glucose on three types of biosensors was recorded and is shown in figure 2 with a potential scan rate of 100 mVs. The results show that all enzyme electrodes have high electron transfer efficiencies. We observed that with an increase in glucose concentration the redox current increased monotonously at a potential higher than 0.4 V and it just became stable only when the applied voltage was higher than 0.6 V. In contrast, the CV curve of a gel-SiO2 modified electrode had an unstable current, and the applied voltage was higher than 0.7 V because of the influence of the oxygen concentration in electrochemical solution. This is important information for applying different immobilization membranes and the mediator. Moreover, we also found that the oxidation current or reduction current increased linearly with the concentration of glucose, and this important result is reported in detail in the next section.

3.4. Amperometric response of glucose sensor

Figure 3 shows the dependence on glucose concentration (0–16 mM) of the CV curves of the electrodes modified by the three immobilizing methods. Obviously, the gelatin/SiO2 modified Pt had the lowest response current and corresponding coefficient ($R^2 = 0.8335$). This indicated that this modified surface had very little immobilized enzyme, thus little $H_2O_2$ was gained in the reaction with glucose. Samples with PB as the electron transfer mediator in PVA-PB-Pt obtained glucose detection sensitivities at 60 $\mu$M ($R^2 = 0.955$). However, the highest response current was obtained with the electrode modified with self-assembled layer of cysteamine ($R^2 = 0.9212$). The modifying chemicals in this case might create a suitable microenvironment that benefits the exposition of the enzyme activity center and increases the response current. This study suggests that the enzyme immobilized on different surfaces has distinct
effectiveness, thus a stable and sensitive glucose sensor may need a combination of the above immobilizing methods.

3.5. Reproducibility and stability of the glucose sensor

The PVA-GOD modified Pt nanowire electrodes were prepared under the same conditions described above for detecting 3 mM glucose. The glucose sensor responses gradually decreased in the first 10 days, the activity remained constant at approximately 60% after 30 days, indicating good stability of the enzyme immobilized on the modified surfaces. Figure 4 shows the decrease in the current response, which is caused by leaking enzyme due to the loose links of the enzyme with the Pt surface after a considerable experiment period.

4. Conclusion

The enzyme immobilization is influenced by linking chemical groups on different Pt surfaces, and the response current of the Pt nanowire based sensor is highly dependent on the utilized surface modification methods. Our research results reveal that GOD immobilized on the Pt nanowires, which were previously modified by PVA with a PB mediator, gave the highest glucose detection sensitivities of about 60 µM. The highest current response was achieved when the Pt nanowires were modified with the cysteamine SAM for subsequent binding of GOD. Furthermore, the stability and catalyst activity of the GOD were retained at about 60% after a store period of 30 days.

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