A Novel Glucose Biosensor Based on Palladium Nanoparticles and Its Application in Detection of Glucose Level in Urine

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A novel glucose biosensor was constructed by electrodeposition of highly dispersed palladium (Pd) nanoparticles on a glassy carbon electrode (GCE). Atomic force microscopy (AFM) was applied to characterize its surface morphology. Electrodeposited Pd nanoparticles exhibited efficiently electrocatalytic oxidation for hydrogen peroxide (H_2O_2) with relatively high sensitivity and stability, which was studied by CV technique and Raman spectroscopy, respectively. The GC/Pd/GOD/Nafion system allowed a low working potential of +0.3 V (vs. SCE). Its signal current was linearly related to the glucose concentration in the range of 1.0×10^{-6}—1.2×10^{-4} mol·L^{-1} with a detection limit of 5.0×10^{-7} mol·L^{-1}. The sensor required no special pretreatment to suppress interference from urate and *L*-ascorbate. It was successfully used in detection of glucose level in human urine with high stability, sensitivity and anti-poisoning ability.

Keywords palladium nanoparticle, electrocatalyze, hydrogen peroxide, glucose oxidase

Introduction

The level of glucose in blood or urine indicates hyper- and hypoglycaemia, both of which can result from a variety of endocrine disorders.[1-4] The rapid and reliable determination of glucose level is a routine project in clinic chemistry. Urine samples are safer and more convenient than blood ones. Meanwhile, the concentration of glucose in serum is closely associated with that in urine.[2-4] Even though glucose electrodes have been successfully used in serum in clinical application, the question still remained of how to detect the glucose level in urine, which has not been widely applied because of the relatively low level of glucose and high interferences in urine samples. Some alternative approaches that overcome this problem were to use thin-film[5] or redox polymer[6] as an enzyme electrode matrix. However, these matrixes were mismatched with the natural environment of enzyme, thus preventing long-term stability and high precision in continuous determination in urine samples.

Nanotechnology offers the possibility of the good support to immobilize enzymes. Nanomaterials were used to improve the stability and catalytic activity of enzyme due to[7,8] (1) higher specific surface area for the binding of a larger amount of enzymes, (2) lower mass transfer resistance and less fouling, and (3) enzyme molecules' orientation would be selectively altered to facilitate the direct charge transfer between glucose oxidase and the electrode.

In this work, it was first reported that electrodeposited Pd nanoparticles showed powerful electrocatalytic activity for the detection of the H_2O_2 liberated from enzymatic reactions. With the effect on Pd nanoparticles, the resulting sensor offered various attractive advantages for monitoring glucose concentration in human urine.

Experimental

Materials

Glucose oxidase (GOD, EC1.1.3.4, 133600 u/g, type VII-s from *Aspergillus niger*), *β-D*-glucose, Nafion (1% methyl alcohol), thymol (methanol solution) and bovine serum album (BSA) were purchased from Sigma. Glutaraldehyde was obtained from Xingzhi Chemical Reagent Company. All other chemicals were of analytical reagent grade. Doubly distilled water was used throughout the experiments. Solutions of $(NH_4)_2PdCl_4$ and K_2SO_4 were freshly prepared every time.

Apparatus

All electrochemical experiments were performed on a CHI 832 electrochemical analyzer (CH Instrument Compa-

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ny, USA). A three-electrode system was used, consisting of a glassy carbon electrode (BAS, $\Phi = 3$ mm) as working electrode, the saturated calomel electrode as reference electrode and the platinum wire as counter electrode.

Atomic Force Microscopy (AFM) was carried out by using an AJ-Ⅲ instrument (Shanghai AJ Nanoscience Development CO. LtD). Raman spectra were recorded using a Super LABRAM confocal microscopic Raman spectrometer (Dilor) with a He-Ne laser (632.8 nm).

Construction of the palladium nanoparticle CME

Prior to the experiment, the glassy carbon electrode (GCE) was polished with 2.0 μm aluminum oxide powder and ultrasonic cleaned sequentially in acetone, nitric acid (1∶1), sodium hydroxide (1 mol·L^{-1}) and doubly distilled water. The GCE was electrochemically activated by potentially scanning in 0.1 mol·L^{-1} K_2SO_4 solution between +1.8 and −0.4 V at a rate of 200 mV·s^{-1}.[9] Then the electrode was cycled from +0.4 to +1.5 V (at 100 mV·s^{-1}) in the modification solution, which contained 5.0×10^{-3} mol·L^{-1} $(NH_4)_2PdCl_4$ in 0.1 mol·L^{-1} K_2SO_4. The modification solution was deaerated for 20 min with pure N_2 before use. After modification, the electrode was rinsed with deionized distilled water for several times. Lastly the CME was cycled in 0.3 mol·L^{-1} H_2SO_4 solution from −0.4 to +1.0 V. N_2 atmosphere was maintained over the solution during the experiment.

Preparation of the enzyme electrode

An enzyme solution containing 229.2 U/mL GOD and 5% BSA was prepared by dissolving approximately 2 mg of GOD and 10 mg of BSA in 0.20 mL of phosphate buffer solution (0.1 mol·L^{-1}, pH 6.9). Then, an aliquot of 50 μL of the enzyme solution and 10 μL of 5% glutaraldehyde solution were mixed thoroughly. Successively, 3 μL of the mixture solution was placed on the electrode modified with Pd nanoparticles. After that, it was cross-linked at room temperature for 2 h. Lastly, 3 μL of 1% Nafion (methyl alcohol solution) was coated on the surface of the GOD electrode. After being air-dried, it was kept in phosphate buffer solution at 4 ℃.

Urine samples

Urine samples were obtained from volunteers, and filtered and diluted (1∶10) in phosphate buffer solution before use. The samples were preserved by adding 0.05% thymol (methanol solution) and kept at 4 ℃ before analysis.

Results and discussion

Prepartion and characterization of Pd *nanoparticles*

The cyclic voltammogram of the electrodeposition of Pd nanoparticles on a glassy carbon electrode (GCE) was shown in Fig. 1. A significant anodic peak was observed at +1.30 V, which indicated that $PdCl_4^{2-}$ was transformed to octahedral Pd^{IV} complex. The CV of the GCE modified with Pd^{IV} in 0.3 mol·L^{-1} H_2SO_4 solution was similar to the reference,[9] where a significant cathodic current arose up around 0.0 V and was attributed to the reduction of Pd^{IV} complex to Pd^0. With the subsequent potential scanning, the CVs reached a steady-state and the electrode surface complex of Pd^{IV} had been almost completely transformed to Pd^0.

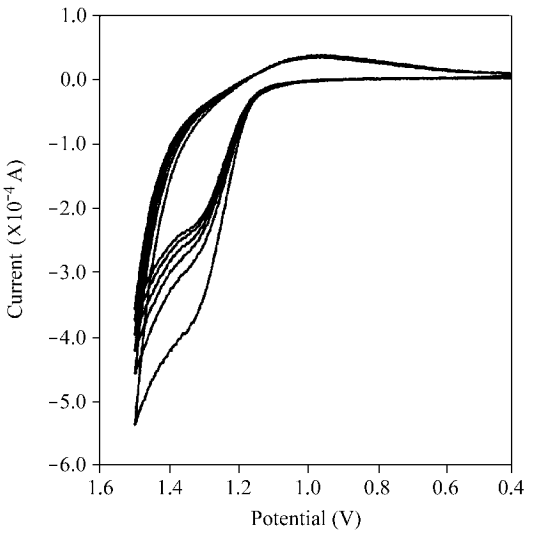

Fig. 1 Cyclic voltammograms of 5.0×10^{-3} mol·L^{-1} $(NH_4)_2PdCl_4$ in 0.1 mol·L^{-1} K_2SO_4 solution between +0.4 and +1.5 V at the scan rate of 100 mV·s^{-1}.

To obtain further insight into the structural features of the Pd CME, the surface of the electrode was characterized by AFM technique. As shown in Fig. 2, Pd nanoparticles were well-dispersed to yield uniformly spherical shape on the GCE surface. The average size of Pd nanoparticles is about 62 nm. The maximum thickness is about 35 nm.

Study on the mechanism of the electrocatalytic oxidation of hydrogen peroxide on Pd *nanoparticle* CME *by* CV *technique and Raman spectroscopy*

Glucose oxidase can catalyze *β-D*-glucose efficiently and selectively. Its products were H_2O_2 and gluconic acid. Hence, the level of glucose may be determined by monitoring the concentration of H_2O_2.

Typical cyclic voltammogram obtained at bare GCE (Fig. 3a) shows that the oxidation of H_2O_2 started close to +0.8 V in agreement with the results obtained in several other studies.[10,11] It is remarkably in contrast with the voltammogram (Fig. 3b) obtained at the Pd-modified electrode at which an appreciable oxidation peak was already observed at +0.2 V. Comparing two voltammograms, the result showed that the overpotential for H_2O_2 lowered more than 0.5 V due to the modification of Pd nanoparticles.

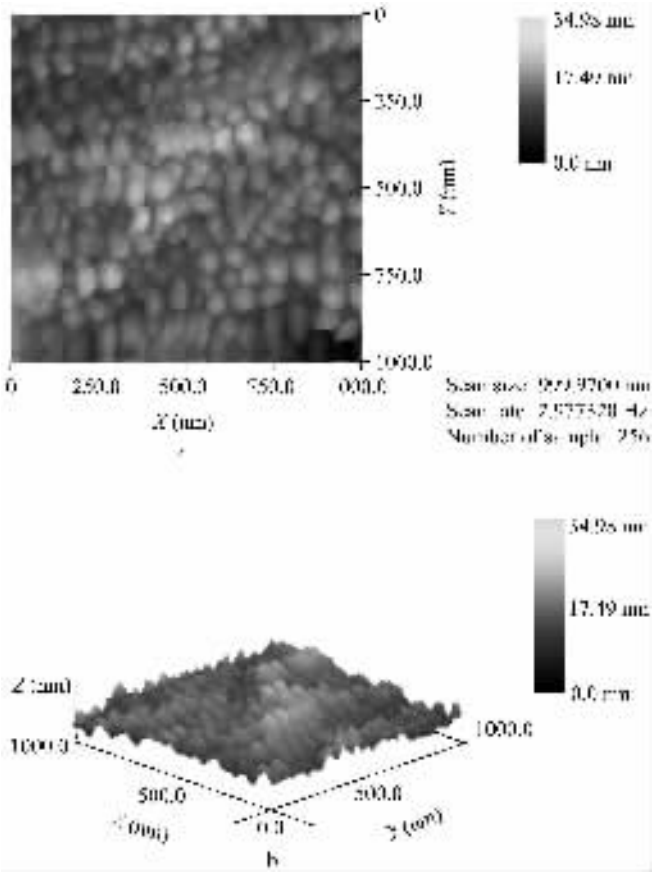

Fig. 2 Tapping mode AFM images of Pd nanoparticle modified GCE surface (a) two-dimensional AFM image; (b) three-dimensional AFM image.

This allowed the application of the comfortably low working potential (+0.3 V), which should serve to minimize the interference from the direct oxidation of other species during practical application of the sensor. Furthermore, its peak current (10^{-3} A) was much larger than that obtained from the bare electrode (10^{-4} A). In addition, the oxidation peak current increased with the increasing concentration of H_2O_2 solution as shown in Fig. 3b. As a result, Pd nanoparticles exhibited a high catalytic activity for the oxidation of H_2O_2, which facilitated the electron transfer between the electrode and the analyte.

The reasonable catalytic mechanism could be expressed as follows:

$$Pd(0) \longrightarrow Pd(IV) + 4e^- \quad \text{(electrode reaction)}$$

$$Pd(IV) + 2H_2O_2 \longrightarrow Pd(0) + 2O_2 + 4H^+ \quad \text{(chemical reaction)}$$

For further support to the mechanism of catalysis, Pd^0 nanoparticle CME was cycled from −0.8 to +1.2 V in 5.0×10^{-4} mol·L^{-1} H_2O_2 solution, then taken out and its Raman spectra were obtained as shown in Fig. 4. According to the reference,[12] the peak located at 427.0 cm^{-1} should be assigned to the characteristic Pd^{IV}. While the

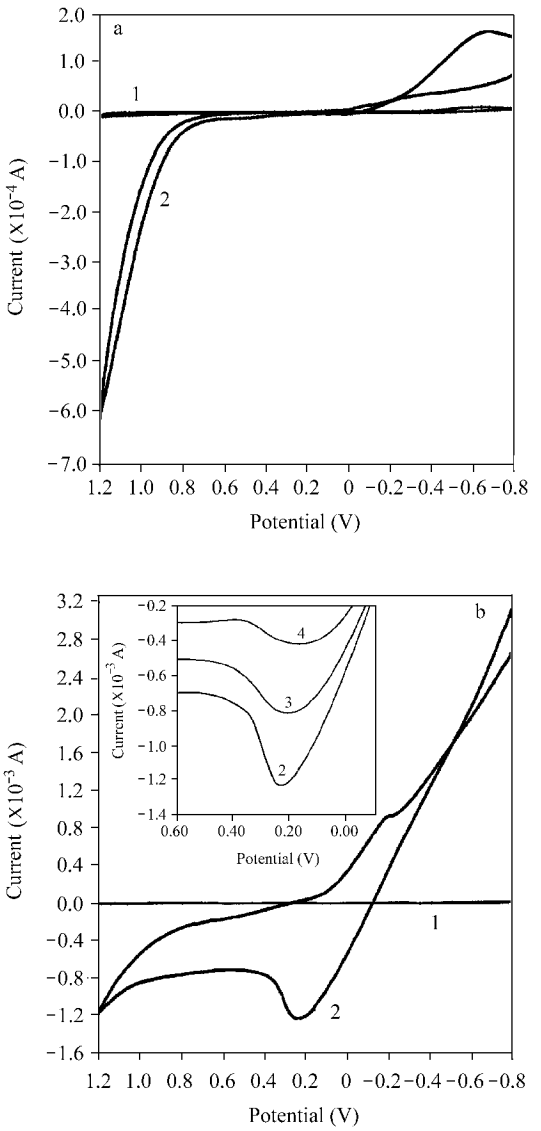

Fig. 3 (a) Cyclic voltammograms for hydrogen peroxide at bare GCE in (1) PBS (phosphate buffer solution); (2) 8.0×10^{-4} mol·L^{-1} H_2O_2 solution; (b) Cyclic voltammograms for hydrogen peroxide at Pd/GCE in (1) PBS solution; (2) 8.0×10^{-4} mol·L^{-1} H_2O_2 solution; (3) 5.0×10^{-4} mol·L^{-1} H_2O_2 solution; (4) 2.0×10^{-4} mol·L^{-1} H_2O_2 solution; Scan rate: 100 mVs^{-1}; electrolyte: 0.1 mol·L^{-1} phosphate buffer (pH = 6.9).

peak at 1042.1 cm^{-1} is red shifted from the characteristic peak of Pd^{IV} (1024.0 cm^{-1}) about 18 cm^{-1}. The reason[13] lies in that the minor diameters of nanoparticles cause the interfacing atoms relatively incompact on the electrode surface, lowing down their vibration frequencies. In addition, no characteristic peak of Pd^{II} (648 cm^{-1})[14] appeared. Therefore, Pd^0 and Pd^{IV} were inter-transformed in this process.

Electrocatalytic response of glucose on GC/Pd/GOD/Nafion *biosensor*

To test the electrocatalytic activity of the GC/Pd/GOD/Nafion biosensor toward glucose oxidation, current-

time responses were obtained at +0.3 V in phosphate buffer solution (pH = 6.9) containing 5.0×10^{-6} mol·L^{-1} glucose by GC/GOD/Nafion and GC/Pd/GOD/Nafion, respectively. As shown in Fig. 5a, the current without modification was only about 10^{-9} A. While with the modified Pd nanoparticles, the current reached to 10^{-7} A (shown in Fig. 5b). Because of their high surface areas,

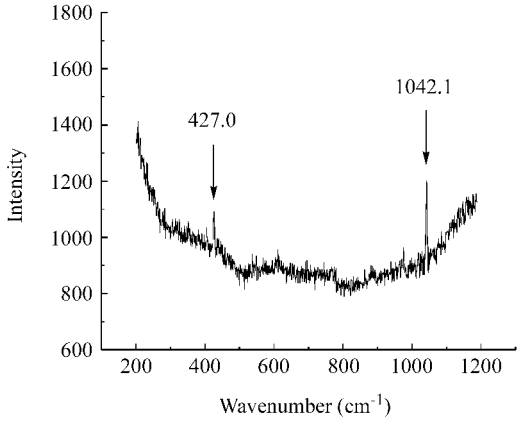

Fig. 4 Raman spectra of Pd modified GC substrate after having cycled from −0.8 V to +1.2 V in 5.0×10^{-4} mol·L^{-1} H_2O_2 solution. Laser power: 5 mW. Acquisition time: 100 s.

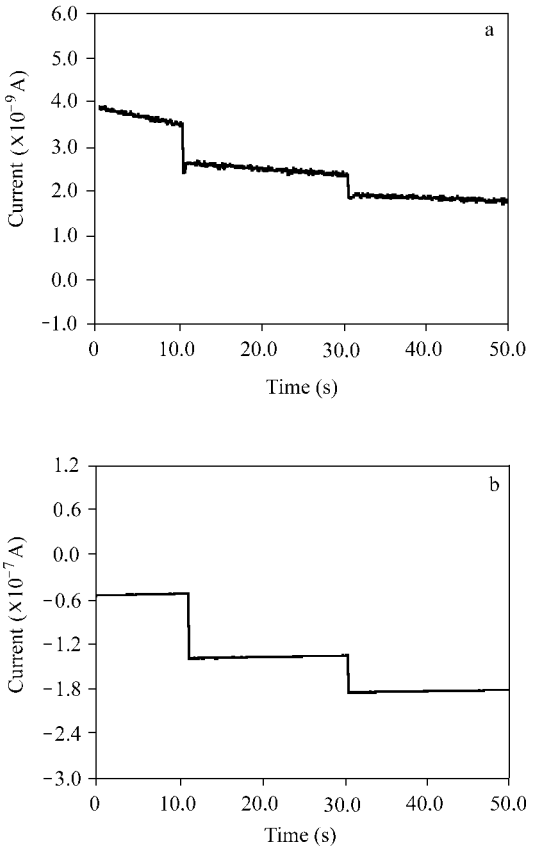

Fig. 5 Current-time response to the added concentration of 5.0×10^{-6} mol·L^{-1} glucose solution at (a) GCE/GOD/Nafion sensor; (b) GCE/Pd/GOD/Nafion sensor, electrolyte: 0.1 mol·L^{-1} phosphate buffer (pH = 6.9); operating potential: +0.3 V (vs. SCE); temperature: 25 ℃.

and multi-active centers, nanoparticles provide a direct charge transfer way between glucose oxidase and the electrode. Furthermore, with the presence of nanomaterials, enzyme molecules' orientation would be altered to facilitate the direct charge transfer.[8] Therefore, Pd nanoparticles greatly enhanced the stability and catalytic activity of glucose oxidase.

The Nafion film[15,16] prevented interferences from diffusion of anions to the surface of the GC electrode. So the sensitivity was increased.

Amperometric response characteristics of the enzyme electrode to glucose

The enzyme electrode was tested by recording the current resulting from consecutive increments of glucose solution in the PBS (each increment to a 5.0×10^{-6} mol·L^{-1} glucose correspondingly). A typical set of response data (at +0.3 V) of a GC/Pd/GOD/Nafion was shown in Fig. 6a. The corresponding calibration curve was illustrated in Fig. 6b. The value of the correlation coefficient (r) was 0.9936. Meanwhile, its response time and detection limit were 15 s and 5.0×10^{-7} mol·L^{-1}, respectively.

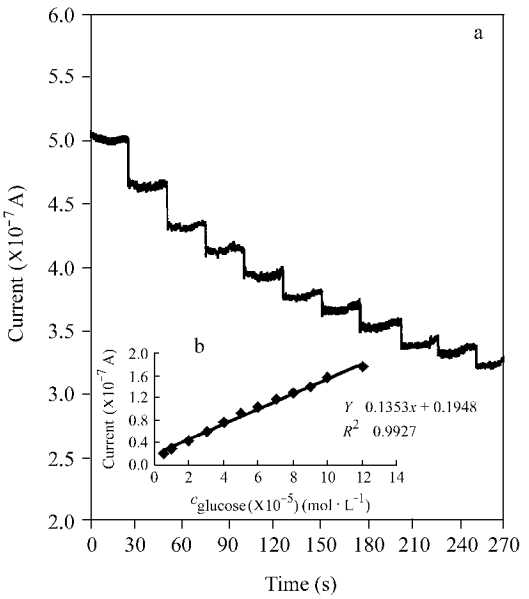

Fig. 6 Typical response curves at GCE/Pd/GOD/Nafion sensor (a) current-time corresponding to successive increment of 5.0×10^{-6} mol·L^{-1} glucose. electrolyte: 0.1 mol·L^{-1} phosphate buffer solution (pH = 6.9); operating potential: +0.3 V (vs. SCE); temperature: 25 ℃; (b) resulting calibration plot.

Effect of temperature

The current response of the biosensor to 5.0×10^{-5} mol·L^{-1} glucose solution in the PBS (pH = 6.9) was measured in the temperature range 15—45 ℃. The results showed that the response increased to reach a maximum level at 35 ℃, and then decreased when the temperature was higher than 40 ℃, at which the enzyme was dena-

tured. Therefore, to take the selectivity and lifetime of the enzyme electrode into account, the room temperature of 25 ℃ was selected for this study.

Operation and storage stability

Operation stability was investigated by consecutive measurement of its response to the same sample within a period of 6 h. After 12 times of measurement, the sensor's activity retained 98% of its initial value. Additionally, the storage stability of the enzyme electrode was tested over four-week period when the electrode was stored in PBS (pH = 6.9) at 4 ℃. No significant decrease in the response to 5.0×10^{-4} mol·L^{-1} glucose solution was found. Good stability may be attributed to the enzyme immobilized strongly with the Pd nanoparticles.

Interference experiments

The interferences of electroactive compounds to the glucose response were examined in the condition of their physiological normal levels in urine with glucose concentration at 5.0×10^{-5} mol·L^{-1}. The influence of ascorbic acid and uric acid could be neglected to the glucose response under the test conditions due to the Nafion film, while glutathione, *L*-cysteine could be skipped due to their low working potential. Moreover, the Pd nanoparticles on GCE prevents the fouling of electrode surface, which enhanced its selectivity and sensitivity in detecting the real samples.

Application in urine samples

A series of five urine samples were assayed in order to demonstrate the practical usage of the biosensor (Table 1). Fresh urine samples were first analyzed in the hospital with the method of $CuSO_4$ reduction by heating, which is the qualitative analysis routinely used in clinical laboratories. These samples were then reassayed with the GC/Pd/GOD/Nafion biosensor. Urine sample (0.5 mL) was diluted with 4.5 mL of PBS (pH = 6.9), and the response was obtained at + 0.30 V. The contents of glucose in urine can then be calculated from the calibration curve. The results are satisfactory and agree closely with those measured by the method of heating $CuSO_4$ reduction[5] in hospital.

Table 1 Determination of glucose level in urine

Sample number	Determined by hospital (10^{-5} mol·L^{-1})	Measured by biosensor (10^{-5} mol·L^{-1})	RSD[b] (n = 5)
1	0.77	0.83	2.06%
2	1.03	1.12	2.74%
3	4.15	4.08	2.37%
4[a]	7.98	7.88	2.98%
5[a]	10.5	10.9	3.52%

[a] represents the hyper level of glucose in urine; [b] RSD = relative standard deviation.

Conclusion

A novel glucose biosensor modified with Pd nanoparticles has been developed. Pd nanoparticles were electrodeposited on a glassy carbon electrode, which exhibited efficiently electrocatalytic activity for the oxidation of H_2O_2 and glucose solution. Then GOD was immobilized on the surface of GC by crossed-linking with BSA and glutaraldehyde solution. Such a glucose biosensor showed a fast response, good operation stability and reproducibility. The work exploited a new method to detect the glucose level in human urine.

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