

Glucose biosensor based on electrodeposited platinum nanoparticles and three-dimensional porous chitosan membranes

Haiwei Chen · Ruo Yuan · Yaqin Chai ·
Jinfen Wang · Wenjuan Li

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Abstract The chitosan with three-dimensional porous structure greatly increased the effective electrode surface for loading of platinum nanoparticles and promoted efficient electron transfer. The resulting biosensor had a response time (within 5 s) and a linear response from 6 μM to 4.2 mM glucose with a detection limit of 2 μM ($S/N = 3$). Moreover, the methodology can be applied for the immobilization of other enzymes.

Keywords Chitosan · Glucose biosensor · Glucose oxidase · Platinum nanoparticles · Porous membranes

Purpose of work A novel and sensitive glucose biosensor based on platinum nanoparticles and glucose oxidase held on a three-dimensional porous chitosan film has been developed.

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H. Chen · R. Yuan (✉) · Y. Chai · J. Wang · W. Li
Chongqing Key Laboratory of Analytical Chemistry,
College of Chemistry and Chemical Engineering,
Southwest University, Chongqing 400715,
People's Republic of China
e-mail: yuanruo@swu.edu.cn

H. Chen
e-mail: w420072c@163.com

Introduction

Glucose biosensors have many potential applications in the food industry and for environmental monitoring and biological analysis, etc. Among the many glucose biosensors that have been developed, amperometric glucose biosensors have played a leading role in blood sugar testing and continuous real time glucose monitoring because of their practical advantages such as operational simplicity, compatibility for miniaturization and low fabrication cost.

With a special interconnected structure and huge surface, three-dimensional porous materials, such as polysaccharides and sol–gels, have attracted increasing attention (Liang et al. 2008). Recently, chitosan-based materials have been extensively applied as immobilization matrices for the fabrication of biosensors (Rubianes and Rivas 2007; Klotzbach et al. 2006), since chitosan has outstanding film forming ability, good water permeability, nontoxicity, biocompatibility and high mechanical strength.

In this work, three-dimensional porous chitosan membranes combining platinum nanoparticles have been applied as immobilization matrices for the fabrication of glucose biosensors. On the one hand, the huge surface and porous structure of the three-dimensional porous chitosan greatly increase the effective electrode surface for platinum nanoparticles loading. On the other hand, platinum nanoparticles have large specific surface area and good biocompatibility for the immobilization of glucose oxidase.

The synergistic action of platinum nanoparticles and three-dimensional porous chitosan improves the electrocatalytic behavior for hydrogen peroxide which is released by the action of glucose oxidase upon glucose, thus, resulting in the improvement of analytical performance of the biosensor.

Materials and methods

Reagents and apparatus

Chitosan (deacetylating grade: 70–85%), glucose oxidase (E.C 1.1.3.4, 157,000 unit/g) and tetraethyl-orthosilicate (97.2% w/w) were purchased from Sigma. β -D-Glucose was from Chongqing Chemical Reagent Co. (China). All other reagents were of analytical grade. Double-distilled water was used throughout this experiment.

Electrochemical measurements were performed at a CHI 660A electrochemical workstation (Shanghai Chenhua Instrument Co., China). The electrochemical cell consisted of a three-electrode system where a modified glassy carbon electrode (diam. = 4 mm) (Lanli Co., Tianjin) was used as a working electrode, platinum wire as an auxiliary electrode and a saturated calomel as a reference electrode. Cyclic voltammetric experiments were performed in 5 ml 5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ containing 0.1 M KCl at 0.05 V/s. Chronoamperometric measurements were performed in a 5 ml 0.1 M phosphate buffer solution (pH 6.5) at 0.6 V. All measurements were carried out at 25°C.

Preparation of chitosan–silica nanocomposites

Silica nanoparticles were prepared with a modification to the method of Liang et al. (2008). Firstly, 10 ml 25% (w/w) NH_4OH and 40 ml ethanol were added to 10 ml double-distilled water with stirring for 10 min at 35°C. Then 6 ml tetraethylorthosilicate was added and the solution was stirred at 300 rpm at 35°C for 24 h. The produced silica nanoparticles were thoroughly washed with water using a neutrality centrifuge and dried at 70°C. To obtain a chitosan–silica nanocomposites suspension solution, 120 mg silica nanoparticles was added to 2 ml 0.5% (w/w) chitosan and mixed using an ultrasonic treatment.

Fabrication of the biosensor

The glassy carbon electrode was firstly polished repeatedly with 0.3 and 0.05 μm alumina slurry, followed by successive sonication in double-distilled water and ethanol for 5 min and dried in air. The chitosan hydrogel with the silica nanoparticles was dropped on the surface of the treated electrode and dried to form a chitosan–silica nanocomposite film at 25°C. It was then treated with 0.05 M glutaraldehyde for 2 h and washed with water. The chitosan–silica nanocomposite modified electrode was finally dipped into 0.05 M HF for 10 min and then washed with water. Subsequently, platinum nanoparticles were electrodeposited on the three-dimensional porous chitosan film in 10 mM H_2PtCl_6 + 0.1 M HCl at -0.2 V. After washing with water, the electrode was immersed in 5 mg glucose oxidase/l overnight at 4°C (shown in Fig. 1). The finished biosensor was stored in 0.1 M phosphate buffer solution (pH 6.5) at 4°C when not in use (Fig. 1)

Results and discussion

Cyclic voltammetry measurements

The cyclic voltammograms of differently modified electrodes are shown in Fig. 2. After the chitosan–silica nanocomposites modified electrode was treated with glutaraldehyde and HF to remove the silica nanoparticles, the redox peak current increased (Fig. 2b). When platinum nanoparticles were electrodeposited on the surface of nanoporous chitosan-modified electrode, the peak current of ferricyanide further increased (Fig. 2c). When glucose oxidase was immobilized on the surface of platinum nanoparticles–chitosan modified electrode, the redox peak current decreased (Fig. 2d), which may be ascribed to the weak conductivity of glucose oxidase (Fig. 2).

Optimization of the experimental conditions

The effect of pH on the biosensor is shown in Supplementary Fig. 1S. Since the optimum pH was 6.5, phosphate buffer solution of pH 6.5 was selected for further experiments.

The effect of applied potential on the biosensor is shown in Supplementary Fig. 2S. Supplementary Section. The response current increased from 0.2 to

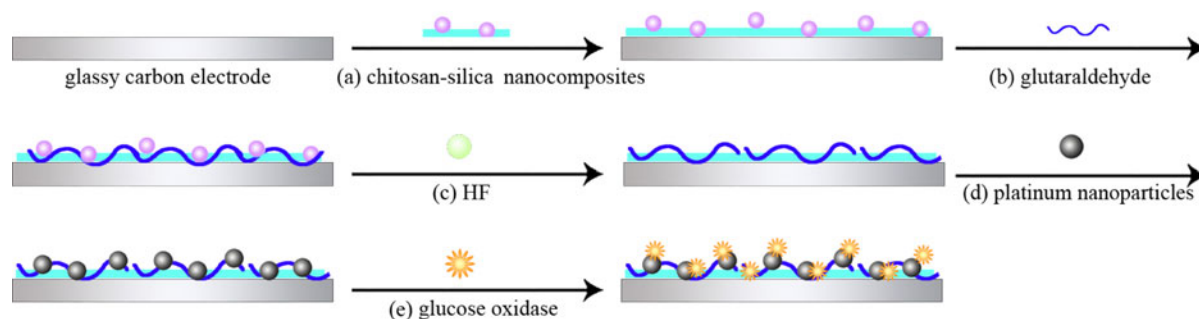


Fig. 1 Preparation process of the biosensor

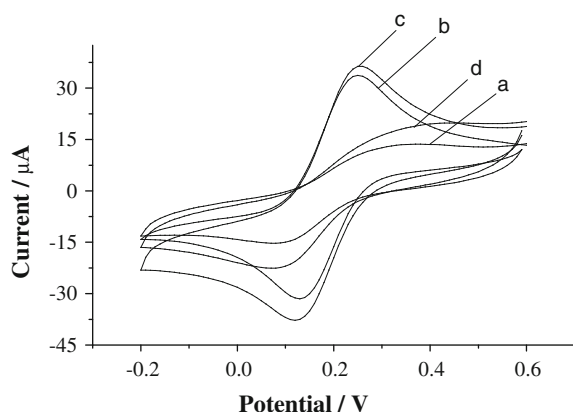


Fig. 2 Cyclic voltammograms of differently modified electrodes: *a* chitosan-silica nanoparticle-modified electrode, *b* chitosan-modified electrode, *c* platinum nanoparticles/chitosan-modified electrode, *d* glucose oxidase/platinum nanoparticles/chitosan-modified electrode. Supporting electrolyte: 5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ + 0.1 M KCl; scan rate, 0.05 V/s

0.6 V. However, a further increase of applied potential led to a decrease of the response current. Therefore, 0.6 V was selected as the operational potential in this work.

The effect of electrodeposition time of platinum nanoparticles on the biosensor was investigated (Supplementary Fig. 3S). The response current of the biosensor increased from 100 to 300 s. However, a further increase of deposition time decreased the response. The response current reached a maximum at 300 s. Therefore, a deposition time of 300 s was selected for the subsequent investigation.

Amperometric response of the biosensor

A typical current–time plot for the biosensor upon the successive addition of glucose at 0.6 V is shown in

Fig. 3. The biosensor reached 95% of the steady-state current within 5 s, which might be ascribed to the three-dimensional porous chitosan and platinum nanoparticles with good conductivity. The inset graph shows the calibration curve of glucose at the biosensor. The biosensor had a linear range from 6 μM to 4.2 mM glucose with a correlation coefficient of 0.9981 ($n = 17$). For comparison, the analytical performance of the proposed biosensor with some other glucose biosensors are listed in Supplementary Table 1S Section (Fig. 3).

When glucose concentration was high, a plateau current appeared, showing the characteristics of Michaelis–Menten kinetics. The K_M^{app} of the proposed biosensor could be obtained from the Lineweaver–Burk equation and was 3.1 mM (Supplementary Fig. 4S), indicating the biosensor had a high affinity to glucose.

Biosensor stability and interference

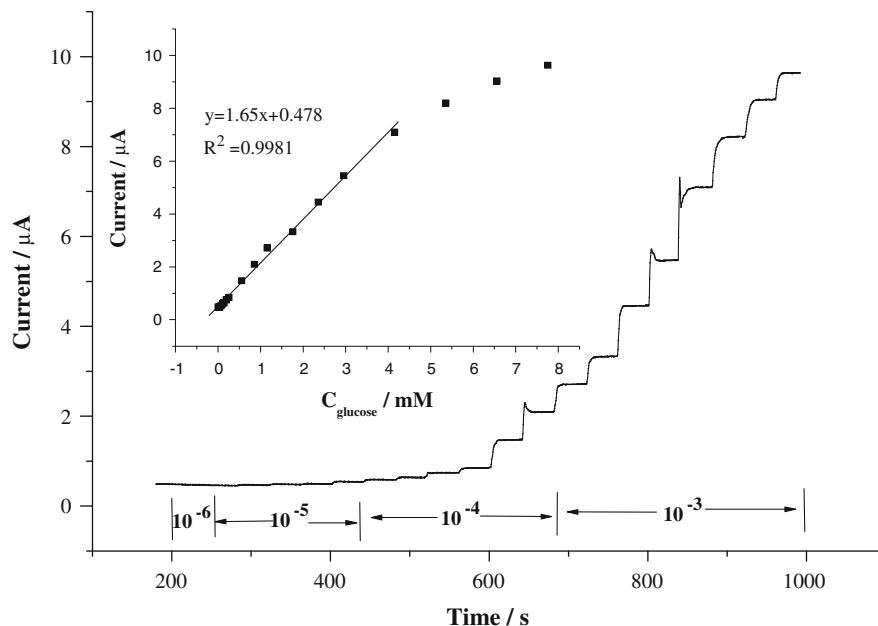
The long-term stability of the glucose biosensor was an important parameter for the evaluation of its performance. When not in use, the electrode was stored in 0.1 M phosphate buffer (pH 6.5) at 4°C and retained 76% of its initial current response for glucose over 2 weeks.

The effects of four possible interferences on the biosensor is shown in Supplementary Fig. 5S. The glucose biosensor had no interference from tryptophan, threonine, leucine or arginine.

Conclusion

A novel strategy for fabricating glucose biosensor based on electrodeposited platinum nanoparticles and

Fig. 3 Typical current–time response curve for successive addition of glucose obtained by the biosensor in stirring 0.1 M phosphate buffer (pH 6.5) at 0.6 V. *Inset graph* calibration curve of the response of the biosensor to glucose in 0.1 M phosphate buffer (pH 6.5)



three-dimensional porous chitosan membranes is proposed. Due to its huge surface and porous structure it is a promising matrix for the fabrication of efficient biosensors. Additionally, the platinum nanoparticles provided a good biocompatible environment and promoted the electrons transfer of glucose oxidase. Moreover, the analytical methodology could be applied for the immobilization of other compounds.

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