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LETTER

Self-assembly of polyaniline-grafted chitosan/glucose oxidase nanolayered films for electrochemical biosensor applications

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Chitosan (CS), an *N*-deacetylated derivative of chitin, is a nature occurring biopolymer found in the exoskeleton of crustaceans, in fungal cell wall, and in other biological materials [1]. It has unusual combination properties [2], which includes excellent membrane-forming ability towards water, good adhesion, nontoxicity, high mechanical strength, especially good biocompatibility and susceptibility to chemical modification due to the presence of reactive amino and hydroxyl function groups. In recent years, chitosan was generally used to immobilize various enzymes. And we have reported the first attempt to prepare chitosan/glucose oxidase (GOD) nanolayered films for electrode modification by the technique of layer-by-layer (LBL) self-assembly [3]. It was found that the response current of the CS/GOD LBL films to glucose was improved since the amount of the immobilization enzyme increased with the increase of the layer number in a certain range. However, chitosan is nearly nonconductive and it hampers the transition of electrons in enzyme multilayer assembly films. Thus, the improvement of the response current was lower than we expected. What's more, the response time gradually extended with the increase of the layer number.

Graft copolymerization of polyaniline (PAN) onto chitosan can enhance the conductivity of chitosan film [4]. In recent years, polyaniline is becoming a promising material for potentiometic biosensor [5–7]. It can increase the sensitivity of the biosensor because the proton generation would occur inside the sensitive layer. However, PAN, like many other conductive polymers, is insoluble in common solvents. Moreover, PAN has rather poor mechanical and general physical properties. In general, the composites obtained by incorporating a rigid conducting polymer (such as PAN) into flexible matrix (such as chitosan) can combine the good processability of the matrix and the electrical conductivity of the conductive polymer [8].

In the present study, chitosan film via graft copolymerization with electroactive PAN was investigated. The modified polymer was subsequently functionalized via layer-by-layer electrostatic self-assembly immobilization of glucose oxidase (GOD). Previous work [4] revealed that surface graft copolymerization of chitosan film with electroactive PAN readily increased the conductivity while remained the hydrophilicity and the biocompatibility. The multilayer membrane is expected to have a rapid response and a better sensitivity simultaneous with a higher response current as the layer number increases.

Chitosan (Mw = 700,000), glucose oxidase (EC1.1.3.4, 340 unit/mg), and glucose were purchased from Sigma Chem. Co. All chemicals were analytical grade. The polymerization to synthesis chitosan grafted with polyaniline was carried out as follows. In a typical reaction [4], 0.2 g chitosan in 40 ml of 2 wt% acetic acid was combined with 40 ml 1 M HCl containing 1 g aniline at 25°C. The solution was cooled to 5°C in an ice/water bath and stirred continuously. Then the 10 ml of HCl solution containing 0.1 g $(NH_4)_2S_2O_8$ was added. The solution was kept at 5°C for 1 h, then the bath was removed and stirring continued for 3 h. Following reaction, the solution was neutralized (to pH ≥ 7) by addition 1 M NaOH solution. The mixture

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was centrifuged at 10,000 rpm for 0.5 h. The supernatant was removed, and the precipitates were washed with deionized water and centrifuged three times. In order to separate the polyaniline from the obtained precipitates, 2 wt% acetic acid was added, followed by removal of the insoluble polyaniline. Afterwards, 1 M NaOH solution was added to the remaining solution and new precipitates were formed. Finally, the resulting solution was centrifuged to obtain polyaniline-grafted chitosan. The resulted precipitates were dried in vacuum.

The preparation of the enzyme electrode was as follows. CS-g-PAN was dissolved in 2% HAc to make 4 mg/ml cationic dipping solution and the glucose oxidase was anionic dipping solution. The surface of the Pt electrode was polished thoroughly with an alumina power and rinsed with distilled water before use. The enzyme immobilization was carried out by a typical self-assembly technique: the Pt electrode was immersed in a CS-g-PAN solution for 20 min, followed by thoroughly rinsing with deionized water for 5 min. The CS-g-PAN-coated substrate was then immersed into a GOD solution for 20 min. The layer-by-layer films of negatively charged GOD and positively charged CS-g-PAN were denoted as $(CS\text{-}g\text{-}PAN/GOD)_n$, where n was the number of bilayer in the films. The resulting enzyme electrode was washed thoroughly with 0.1 M phosphate buffer solution (PBS), and then stored in PBS with pH 6.8 at 4°C. The layer-by-layer deposition of CS-g-PAN and GOD was also proved by QCM as we have reported in our previous work [3].

The determination principle of the current response is based on the formation of hydrogen peroxide during the enzyme-catalyzed reaction. The hydrogen peroxide is detected by amperometric method. The electrolysis cell consisted of a CS-g-PAN/GOD-coated Pt working electrode, a Pt counter electrode, and Ag/AgCl as a reference electrode. The electrochemical process was carried out in 0.1 M PBS (pH 6.8) at a constant potential of 0.60 V. The apparatus used for determining the current response was Potentiostat/Galvanostat (Model 283, EG&G PARC with software M270).

FT-IR spectra were used to characterize polyaniline-grafted chitosan. FT-IR spectra were recorded on a BIO-RAD FTS3000 FT-IR spectrometer. Spectra were taken with a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}. Samples were thoroughly ground with exhaustively dried KBr and discs were prepared by compression under vacuum.

The infrared spectra of chitosan and polyaniline-grafted chitosan are shown in Fig. 1. In the spectrum of the chitosan (Fig. 1(a)), distinctive absorption bands at 3421 cm^{-1}, 2811 cm^{-1} ($-NH_2$ stretching) and 1647 cm^{-1}, 1597 cm^{-1}, 1380 cm^{-1} ($-NH_2$ bending) are found. The absorption bands at 1152 cm^{-1} (anti-symmetric stretching of C–O–C bridge), 1088 cm^{-1}, 1035 cm^{-1} (skeletal vibration involving the C–O stretching) are characteristics of its saccharide structure. The new absorption bands at 3404 cm^{-1}, 754 cm^{-1} (–NH bands) and 1563 cm^{-1}, and 1412 cm^{-1} (phenyl bands) can be detected from grafted chitosan IR spectrum (Fig. 1(b)). In addition, the intensity of the bands of $-NH_2$ is decreased, which suggests that polyaniline has been successfully grafted onto chitosan backbone [4].

Figure 2 shows the amperometric current response of the enzyme electrodes modified by CS-g-PAN/GOD (curve a) and CS/GOD (curve b), respectively, as a function of layer number. Note that the response current of the two kinds of enzyme electrodes enhances since the amount of the immobilized GOD increases with the increase of the film's layer number. Whereas chitosan film via graft

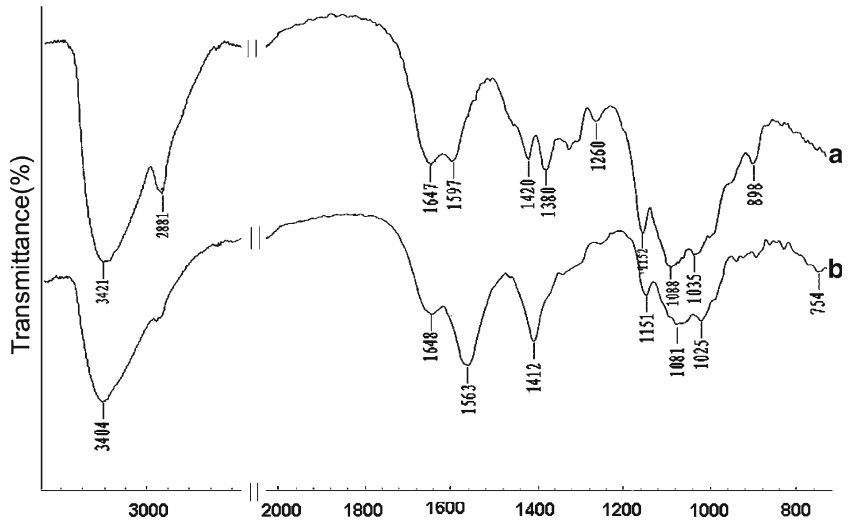


Fig. 1 Infrared spectrum of (a) chitosan and (b) polyaniline-grafted chitosan

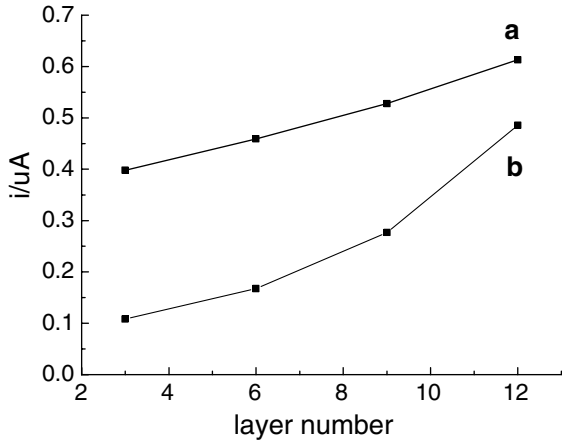


Fig. 2 Effect of layer number of self-assembly films on the current response to 5 mM glucose

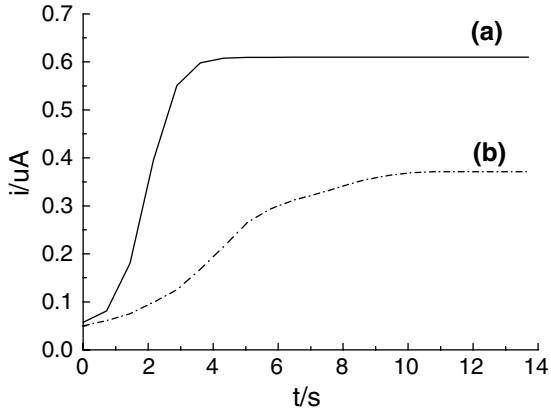
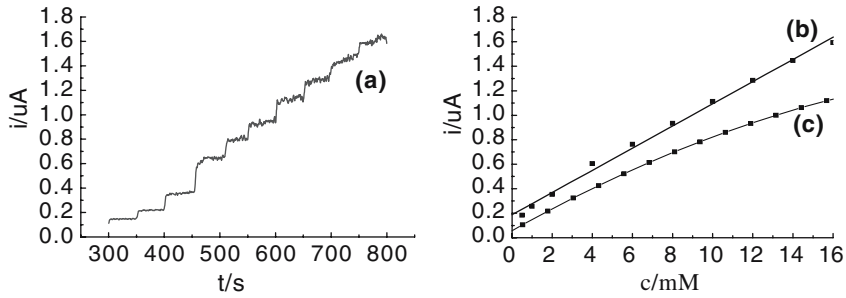


Fig. 4 The steady-state current response time of GOD biosensor covered with $(CS\text{-}g\text{-}PAN/GOD)_{12}$ (a) and $(CS/GOD)_{12}$ (b), respectively, to 5 mM glucose

Fig. 3 Current response of the $(CS\text{-}g\text{-}PAN/GOD)_{12}$ biosensor in the concentration range of 0.5–16 mM (a), the linear calibration plot of enzyme electrodes modified by $(CS\text{-}g\text{-}PAN/GOx)_{12}$ (b) and $(CS/GOx)_{12}$ (c), respectively



copolymerization with electroactive PAN enhances the ability of electron transition. Therefore, compared with CS/GOD, the CS-g-PAN/GOD multilayer films can improve the current response more effectively.

Figure 3a displays a typical current-time response using the enzyme electrode under the optimal experimental conditions (pH 6.8, working potential 0.6 V) after the addition of successive aliquots of glucose to PBS. A well-defined reduction current proportional to the glucose concentration has been observed. In Figure 3, curve (b) $[(CS\text{-}g\text{-}PAN/GOD)_{12}]$ and curve (c) $[(CS/GOD)_{12}]$ show the amperometric current response of the biosensor as a function of glucose concentration. The response current increases linearly with glucose concentration from 0.5 mM to 16 mM for the $[(CS\text{-}g\text{-}PAN/GOD)_{12}]$ and from 0.5 mM to 8 mM in the case of $[(CS/GOD)_{12}]$. The $(CS\text{-}g\text{-}PAN/GOD)_{12}$ biosensor has a broader linear range and higher response current than $(CS/GOD)_{12}$ biosensor, which can satisfy the detection of glucose in human body.

Figure 4 illustrates the steady-state current response time of the two biosensors with CS-g-PAN/GOD (a) and CS/GOD (b) under identical condition. Evidently, the former shows a faster response than the latter (5.2 s vs. 10.1 s). Furthermore, the biosensor with CS-g-PAN/GOD has a greater response current than its counterpart. Due to the conductivity of PAN, CS-g-PAN film has a faster transition of electron than CS film. Accordingly, it can enhance the output current and reduce the time of response.

In conclusion, we introduced a new type of amperometric GOD biosensor based on CS-g-PAN/GOD, which was prepared by the technique of layer-by-layer electrostatic self-assembly. The glucose biosensors fabricated using the CS-g-PAN/GOD multilayer film exhibited a faster response and a higher output current to glucose in the normal and diabetic level.

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References

1. Gpeter M (1995) Pure Appl Chem A32:629
2. Chandy T, Sharma CP (1990) Biomater Art Cells Art Org 1:18
3. Xu XH, Han B, Han J et al (2003) J Mater Sci Lett 22(9):695
4. Yang S, Tirmizi SA, Burns A et al (1989) Synthetic Met 32:191
5. Oksana AK, Bobrova A, Lukachova LV et al (1996) Sensor Actuat B-Chem 33:34
6. Ivanov AN, Lukachova LV, Eytugyn GA et al (2002) Bioelectrochemistry 55(1–2):75
7. Xue HG, Shen ZQ, Li YF (2001) Synthetic Met 124(2–3):345
8. Kim SJ, Shin SR, Spinks GM (2005) J Appl Polym Sci 96:867