Bioelectrochemistry 88 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Biocompatibility of CS–PPy nanocomposites and their application to glucose biosensor

Yi Fang [a], Yalong Ni [a], Guohui Zhang [a], Chun Mao [a,*], Xiaohua Huang [a,*], Jian Shen [a,b]

[a] Jiangsu Key Laboratory of Biofunctional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, PR China
[b] Research Center of Surface and Interface Chemistry and Engineering Technology, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China

ARTICLE INFO

Article history:
Received 16 February 2012
Received in revised form 26 May 2012
Accepted 30 May 2012
Available online 7 June 2012

Keywords:
Polypyrrole
Chitosan
Nanocomposites
Glucose oxidase
Biosensor

ABSTRACT

The intrinsic properties and application potential of nanocolloids are mainly determined by size, shape, composition, and structure. In this case, a novel glucose biosensor was developed by using the chitosan–polypyrrole (CS–PPy) nanocomposites as special modified materials that coating onto the surface of glassy carbon electrode (GCE). The CS–PPy nanocomposites were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM), respectively. Moreover, the interaction of CS–PPy nanocomposites with glucose oxidase (GOD) was also investigated by the combined studies with Fourier transform infrared spectroscopy (FTIR) and circular dichroism spectroscopy (CD). Due to the conductivity of polypyrrole (PPy), good biocompatibility of CS, and advantages of nanoparticles, CS–PPy nanocomposites were chosen and designed to modify the GCE for the retention of GOD's biological activity and the vantage of electron transfer between GOD and electrodes. The GOD biosensor exhibited a fast amperometric response (5 s) to glucose, a good linear current–time relation over a wide range of glucose concentrations from 5.00×10^{-4} to 1.47×10^{-1} M, and a low detection limit of 1.55×10^{-5} M. The GOD biosensor modified with CS–PPy nanocomposites will have essential meaning and practical application in future that attributed to the simple method of fabrication and good performance.

1. Introduction

A biosensor, by definition requires stable and reproducible immobilization of a biological entity (e.g. vitamins, coenzymes, proteins, DNA, polypeptides, cells, microorganisms) on a transducer surface, with complete retention of its biological activity [1–8]. Compared with free enzymes in solution, the immobilized enzymes are more robust and have the advantages, such as convenient handling, easy separation from the product, operational stability, more resistant to environmental changes and feasibility of the reuse [9]. Many methods have been developed for the immobilization of enzymes, such as the attachment of them to the prefabricated carriers by adsorption, covalent bonds, or ionic bonds, cross-linking with other multifunctional reagents and encapsulation or inclusion in membranes or sol–gel matrices [10–14]. In the past few years, various materials have been used as immobilization matrices and enhance the efficiency of electrical conductivity, such as Au, Ag, Ag–Au, copper and platinum nanoparticles [15–19], titania nanocomposite [20], carbon nanotube[21], nickel magnetic nanoparticles [22], NiO nanocomposite [23], semiconductor nanoparticles (CdSe/ZnS) [24] and so on.

Conducting polymers (polypyrrole, polyaniline, polyacetylene, etc.) have been widely used as matrices of biomolecules [25–31], because the controlled growth of such molecules could satisfy different requirements such as polymer layer thickness, electrical properties and bio-reagent loading, etc. Therefore, conducting polymer based biosensors can be used in *in vivo* sensing, continuous monitoring of drugs or metabolites, multi-parametric assays, miniaturization and high information density [32].

However, the poor processability of conducting polymers remains a key hurdle to their practical application. The dispersion of conducting polymers composed of low-dimensional nanostructures including nanoparticles, nanofibers and nanotubes has offered the possibility of improving the processability of these interesting polymers [33]. Rajesh et Al. gave an overview of various recent synthetic approaches involving template free and template oriented techniques suitable for the growth of nanomaterials of conjugated polymers, their merits and application in making nanodevices. He pointed out that conducting polymeric nanomaterials are found to have superior performance relative to conventional materials due to their much larger exposed surface area. Moreover, these materials are promising for a variety of applications including optical and electronic nanodevices, and chemical and biological sensors. Novel nanomaterials for use in bioassay applications represent a rapidly advancing field [34].

As functional materials, chitosan (CS) offers a unique set of characteristics: biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, antibacterial properties, gel forming properties and hydrophilicity, and remarkable affinity to proteins [35–42]. Owing to these characteristics, chitosan-based materials, as yet

* Corresponding authors. Tel.: +86 25 8589 1536.
E-mail addresses: maochun127@yahoo.cn (C. Mao), huangxiaohua@njnu.edu.cn (X. Huang).

1567-5394/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2012.05.006

underutilized, are predicted to be widely exploited in the near future especially in environmentally benign applications in systems working in biological environments, among others as enzyme immobilization supports [35].

In this case, in order to combine the conductivity of polypyrrole (PPy), biocompatibility of CS, and advantages of nanoparticles, CS–PPy nanocomposites were chosen and designed to modify the glassy carbon electrodes (GCEs) for the retention of glucose oxidase's (GOD) biological activity and the electron transfer between GOD and electrodes. By immobilizing GOD onto the matrix of CS–PPy nanocomposites coated on the surface of GCE, a novel glucose biosensor was constructed and its electrochemical behavior was investigated in detail.

2. Experiments

2.1. Reagents

Glucose oxide (GOD) was purchased from Sigma-Aldrich (USA), β-D-(+)-glucose (99%) was obtained from J&K Chemical Co. Inc. (China), chitosan was obtained from Nantong Shuanglin Biological Co. Ltd. (China), pyrrole (99%) was received from ACROS Organics Co. Ltd. (USA), glutaraldehyde (25%) was purchased from Shanghai Lingfeng Chemical Co. Ltd. (China) and (3-Amincpropyl)-triethoxysliane (APTES) (98%) was purchased from Aladdin Chemistry Co. Ltd. (China). All the above reagents were used without further purification. Phosphate buffer solution (PBS) was prepared by mixing stock standard solution of Na_2HPO_4 and NaH_2PO_4. All other chemicals were of analytical grade and were used as received. All solutions were prepared with double-distilled water and high purity N_2 was applied for deaeration.

2.2. Preparation of CS–PPy nanocomposites

The CS–PPy nanocomposites were prepared according to the literature [43]. Firstly, 0.15 g CS was dissolved in 30 mL 0.25% Acetic acid (HAc, mass concentration ratio) water solution and 0.90 g $FeCl_3 \cdot 6H_2O$ was added into the CS aqueous solution under stirring until it was dissolved completely. Secondly, 100 μL of pyrrole agent ($FeCl_3$/Py = 2.33, the concentration ratio of CS/Py were 1:3, 1:6 and 1:9) was added into the above solution slowly under 0–5 °C ice water bath condition, the color of solution quickly turned into black. After the mixed solution was further stirred for 1 h, the CS–PPy nanocomposites were obtained. Then the sample was dialyzed with Spectra/Por CE (MWCO = 2000) in 0.25% HAc water solution for three days to thoroughly remove iron ion. The resulting CS–PPy nanocomposites were used for characterization and detection. The prepared nanocomposites were stable at 4 °C even after 6 months of storage.

2.3. Construction of the GOD/CS–PPy/glutaraldehyde/APTES modified electrode

Prior to the modification, the GCE was successively polished to a mirror-like surface with 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with double-distilled water. After sonicated in ethanol and double-distilled water for 5 min respectively, the GCE was dried at room temperature.

As shown in Scheme 1, 4.0 μL of 1.0% APTES solution (anhydrous alcohol as solvent) and 4.0 μL of 0.25% glutaraldehyde aqueous were successively dropped onto the electrode surface and dried in air. APTES was linked to the GCE surface through the silicon–oxygen bonds. And the aldehyde group of glutaraldehyde combined with the amino at the end of APTES molecule. After that, 8.0 μL of the obtained CS–PPy nanocomposites solution was dropped to the surface of modified GCE and dried in room temperature. Another aldehyde group of glutaraldehyde was reacted with the amino group of CS. Finally, the fresh prepared mix solution of 8.0 μL of 2 $mg \cdot mL^{-1}$ GOD solution in PBS (pH = 7.0) and 2.0 μL of 0.25% glutaraldehyde aqueous was dropped onto the surface of CS–PPy/glutaraldehyde/APTES/GCE and kept overnight at 4 °C, then the GOD was immobilized on the electrode, and GOD/CS–PPy/glutaraldehyde/APTES/GCE was obtained. The other electrodes used as contrast samples were prepared by the same modified method. When not in use, the electrodes were stored at 4 °C in a refrigerator.

2.4. Apparatus and measurements

Scanning electron microscopy (SEM) images were recorded on a JSM Model 6300 scanning electronic microscopy. Transmission electron microscopy (TEM) images were obtained using an interface high-resolution transmission electron microscopy (HITACHI H-7650, Japan). FTIR spectra of CS–PPy nanocomposites, GOD and GOD/CS–PPy nanocomposites were measured using a Cary 5000 Fourier transform infrared (FTIR) spectrophotometer (VARIAN Company). CD spectra in the far-UV (with the range from 200 to 260 nm) were measured on a JASCO J-715 spectropolarimeter using a 1 cm quartz cuvette. The contents of α-helix, β-sheet, β-turn and the random coil conformation were calculated using the JASCO710 program. All electrochemical experiments were performed on a CHI 760C electrochemical analyzer (CH Instruments, Inc., US), using a conventional three-electrode system with an glassy carbon electrode (GCE) (3 mm in diameter, Shanghai Chenhua, China) as the working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. Cyclic voltammogram (CV) experiments were carried out in quiescent solution at 100 $mV \cdot s^{-1}$ in 5 mL of 0.1 M PBS, and the solution was purged with high purity nitrogen prior to and blanked with during the electrochemical experiments.

3. Results and discussion

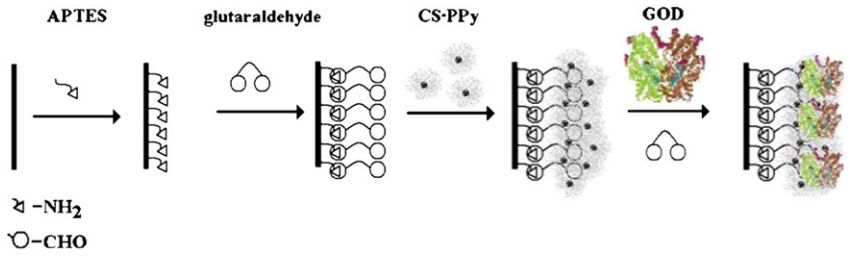
3.1. Characterization of the CS–PPy and GOD/CS–PPy nanocomposites

CS has been widely used as biomedical material due to its good biocompatibility [37,39], while PPy is a representative of conducting polymers, which have attracted much interest in recent years [25–31]. Combing the characteristics of both materials, CS–PPy nanocomposites can be potentially advantageous in both biocompatibility and conductivity.

Fig. 1A shows the TEM image of the CS–PPy nanocomposites. It could be observed that the PPy nanoparticles (in black) were hybridized and well-dispersed in reticular CS (in gray) polymer. The PPy nanoparticles are spherical in shape with the average size of 75 nm.

The surface morphology of modified electrode was further confirmed by SEM. Compared with the smooth surface of unmodified electrode (Fig. 1B), the modified electrode prepared by coating CS–PPy/glutaraldehyde/APTES behaves quite differently. In the SEM image (Fig. 1C) of the modified electrode, PPy nanoparticles assembled together to form a microstructured surface containing many pores. The CS–PPy nanocomposites and the pores showed a three-dimensional micro-nano topological structure, which might promote the immobilization of enzyme [44].

The fabricated products of the CS–PPy nanocomposites stabilized GOD was further investigated by FTIR spectroscopy. Fig. 2A shows the FTIR spectra of the CS–PPy (a), pure GOD (b) and GOD/CS–PPy (c), respectively. Three strong peaks at 1398 cm^{-1}, 3039 cm^{-1} and 3129 cm^{-1} were observed on the spectrum of CS–PPy nanocomposites. The two peaks at 1398 cm^{-1} (C-OH stretch), 3039 cm^{-1} (C–H stretch) are characteristic peaks of CS–PPy, and the peak at 3129 cm^{-1} corresponds to the N–H stretch of amine and O–H stretch of hydroxyl groups [45]. In the case of GOD, the most important features are the amide band (the peaks around 1660 cm^{-1}) and the amide II band (the peaks around 1553 cm^{-1}) of the amide group [46,47]. The two amide bands are distinguishing features for the secondary structure of the polypeptide



Scheme 1. The process of the GOD/CS–PPy/glutaraldehyde/APTES modified electrode.

chain. By comparing the spectra of pure GOD (B) and GOD/CS–PPy blend film (C), the peak positions were similar, except that the intensity is lower when GOD immobilized on CS–PPy nanocomposites. This phenomenon can be explained by the hypothesis that GOD was trapped in CS–PPy nanocomposites efficiently and retains high enzyme activity.

Due to its high sensitivity to polypeptide backbone conformations, circular dichroism was used to study the secondary and tertiary structure of the protein [48]. Fig. 2B shows the CD spectrum of GOD in the PBS without and with CS–PPy nanocomposites in the far-UV region. The positive bands at 203 nm in both curves correspond to the π–π* transition of the amide groups in the GOD peptide chain. Two negative bands at 209 and 219 nm are in accordance to the π–π* and n–π* transition of the amide groups of the GOD polypeptide chain, respectively. In the presence of CS–PPy nanocomposites, the intensity of the positive band at 203 nm significantly decreases compared with that of pure GOD, indicating the interactions between the amino acid residues of GOD and CS–PPy nanocomposites. Additionally, the intensity of the dual bands at 209 and 219 nm had a slight increase for the GOD/CS–PPy, indicating that the secondary structure of GOD was not obviously changed.

By comparing with the data calculated from CD spectrum, it can be observed that GOD secondary structure undergoes certain changes in the presence of CS–PPy nanocomposites. The content of α-helix conformation decreased by 1.8%; while the contents of anti-parallel, β-turn and random coil increased by 0.2%, 0.3% and 0.7%, respectively. These results further proved that CS–PPy nanocomposites did not destroy the native conformation of GOD, which also demonstrated high biocompatibility of the CS–PPy nanocomposites.

3.2. Evaluation of the electrochemical performance of the glucose biosensor

Cyclic voltammetry (CV) was used to evaluate the electrochemical performance of the electrodes. Fig. 3 shows CVs of different modified electrodes of GOD on CS–PPy nanocomposites in PBS (pH = 7.0) at 100 $mV \cdot s^{-1}$. No voltammetric signal was observed at bare glassy carbon electrode (GCE) (curve (A)), glutaraldehyde/APTES/GCE electrode (curve (B)) and CS–PPy/glutaraldehyde/APTES/GCE electrode (curve (C)) in the range of −0.2 to 0.4 V vs. SCE. When GOD was embedded in the glutaraldehyde/APTES/GCE electrode (curve (D)), the obtained CV curve is almost flat, just a pair of little parcels could be observed at about 0.22 V vs. SCE. The two little parcels are oxidation-reduction peaks of GOD. The small peaks mean that electron transfer of GOD on glutaraldehyde/APTES/GCE is very slow. When GOD was embedded in the CS film (curve (F)), the observed oxidation-reduction peaks on GOD/CS/glutaraldehyde/APTES/GCE electrode are more clearly compare with that of on CS/glutaraldehyde/APTES/GCE (curve (E)). This demonstrated that the good biocompatibility of CS could provide the favorable

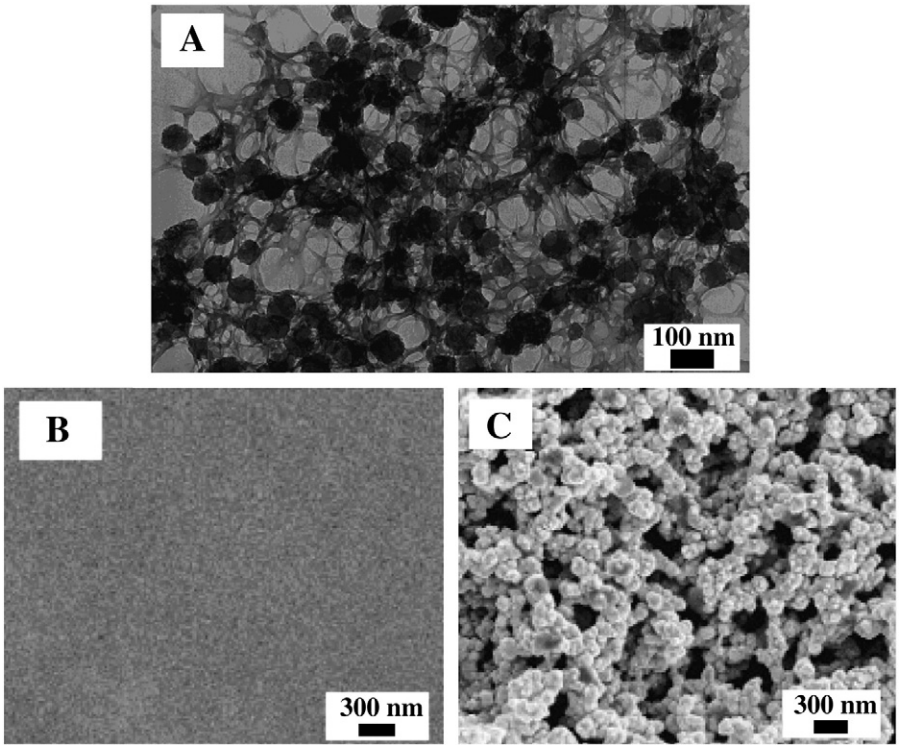


Fig. 1. (A) TEM image of the CS–PPy nanocomposites. (B) SEM image of the surface of unmodified electrode. (C) SEM image of the surface of modified electrode prepared by coating CS–PPy/glutaraldehyde/APTES.

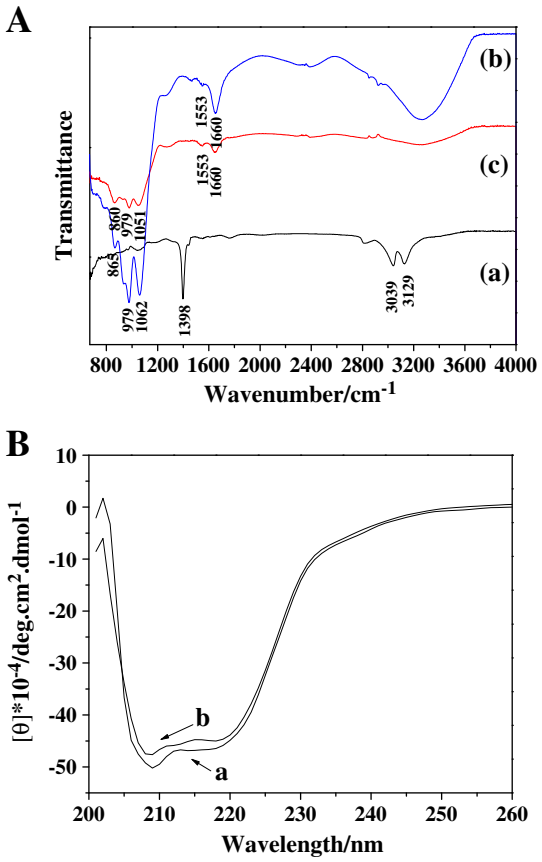


Fig. 2. (A) FTIR of (a) CS–PPy; (b) pure GOD and (c) GOD/CS–PPy. (B) The CD spectra of (a) 1.56×10^{-5} M GOD and (b) GOD/CS–PPy in the PBS (pH = 7.0) in the wavelength region of 200–260 nm.

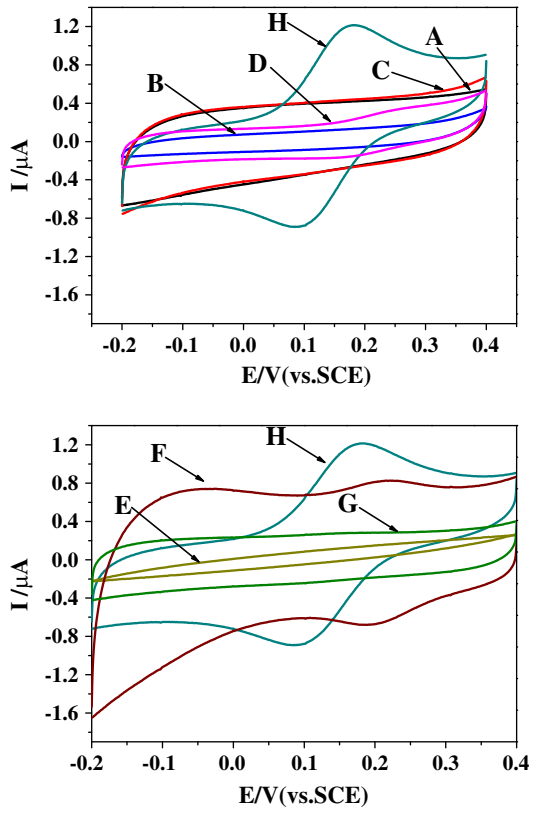


Fig. 3. Cyclic voltammograms (CVs) of different modified electrodes in 0.1 M PBS (pH = 7.0) containing 0.1 M KCl for (A) bare GCE electrode; (B) the glutaraldehyde/APTES/GCE electrode; (C) the CS–PPy/glutaraldehyde/APTES/GCE electrode; (D) the GOD/glutaraldehyde/APTES/GCE electrode; (E) the CS/glutaraldehyde/APTES/GCE electrode; (F) the GOD/CS/glutaraldehyde/APTES/GCE electrode; (G) the GOD/CS–PPy (1:9)/glutaraldehyde/APTES/GCE electrode; (H) the GOD/CS–PPy (1:6)/glutaraldehyde/APTES/GCE electrode (scan rate is 100 $mV\cdot s^{-1}$).

microenvironment for GOD. From the CVs of CS/glutaraldehyde/APTES/GCE and GOD/CS/glutaraldehyde/APTES/GCE, we can get the result that the observed oxidation and reduction peaks come from GOD, not CS. However, only when GOD was embedded in the matrix that containing the CS–PPy nanocomposites (curve (H)), a pair of well-defined oxidation and reduction peaks could be observed with a formal potential (E°′) at about 0.132 V vs. SCE. This biosensor based on nanocomposites showed a very stable electrochemical response even after 500 cycle measurements, which made it suitable for further investigations and applications.

Apparently, the current response of CVs increased when CS–PPy nanocomposites were assembled into the glutaraldehyde/APTES membrane (curve (G)) indicated that the CS–PPy nanocomposites promoted electron-transfer reactions of GOD. CS may provide a native environment for the enzyme molecules to orient in conformations more favorable for electron transfer [45]. On the other hand, PPy could provide a conductive path through the composite membrane matrix. The effect of different concentration ratio (1:3, 1:6 and 1:9) of CS/PPy on conductive behavior was investigated. Addition of PPy improved the electrode conductivity and made the peak separation potential increased. When concentration ratio of the CS/PPy is at 1:6 (curve (H) in Fig. 3), the increased value of peak separation potential was in the permitted extend of quasi-reversible oxidation-reduction process, a pair of well-defined oxidation and reduction peaks could be obtained, so the CS/PPy ratio of 1:6 is appropriate for electrochemical sensing application. However, if the PPy concentration was too high (CS/PPy ratio is at 1:9) or even there is no CS on electrode surface, the electrochemical activity of the enzyme could not express normally (curve (G) in Fig. 3). This is because enzyme lost its activity that can be attributed to PPy could not provide biocompatible environment for enzyme. In this system, the actual role of PPy is to enhance the conductivity of the electrode, so that good electrochemical sensing of biosensor can be obtained from appropriate proportion of CS/PPy. The results of the CVs proved that CS–PPy, acting as electron-transfer mediators, can help in enhancing the current response of enzyme electrode and effective increasing the sensitivity of the biosensor. It's worth noting that the CS–PPy nanocomposites have a sort of three-dimensional micro-nano topological structure, which may also increase the loading amount of the bioactive enzymes.

As is well known, β-D-glucose, the substrate of GOD, will result in the reductive form of GOD (GOD-$FADH_2$) in the enzyme-catalyzed reaction. The electron-transfer behavior of the GOD on CS–PPy/glutaraldehyde/APTES/GCE occurs as the following [49]:

$$GOD - FAD + 2e^- + 2H^+ \leftrightarrow GOD - FADH_2 \quad (1)$$

$$Glucose + GOD - FAD \rightarrow gluconolactone + GOD - FADH_2 \quad (2)$$

$$GOD - FADH_2 + O_2 \rightarrow GOD - FAD + H_2O_2 \quad (3)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 + 2e^- \quad (4)$$

A typical current–time plot (Fig. 4A) of electrocatalytic oxidations of glucose by using the prepared glucose biosensor was obtained under the optimized experimental condition. The experiments showed that the electrocatalytic response was very fast; the biosensor achieved 95% of the maximum steady-state response current to glucose in less than 5 s, which demonstrated that the CS–PPy nanocomposites might provide a good micro-environment for GOD so that the redox system shows fast electrode kinetics. The resulting

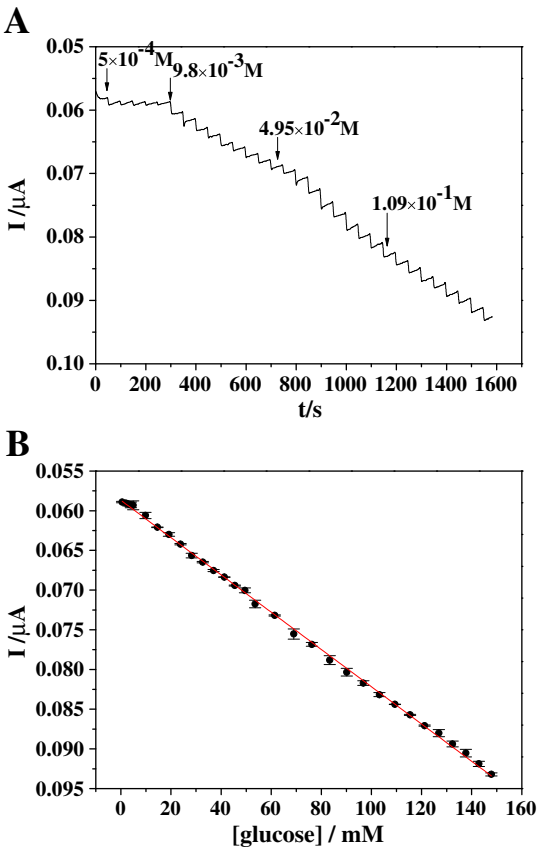


Fig. 4. (A) Amperometric response of the glucose biosensor of successive addition of different concentration of glucose 0.1 M PBS (pH = 5.5) at the working potential of +0.132 V. (B) The calibration plot between the current and the concentration of glucose.

calibration plot of current–time response of GOD/CS–PPy/glutaraldehyde/APTES/GCE is presented in Fig. 4B. The catalytic oxidation peak current increased linearly with the increasing concentration of glucose in a range from 5.00×10^{-4} to 1.47×10^{-1} M, and the linear regression equation is $i(\mu A) = 0.0587 + 2.3466\times10^{-4}\,C$ (M) ($R^2 = 0.9992$) with a detection limit of 1.55×10^{-5} M (S/N = 3).

The glucose biosensor showed a quite good anti-interference ability (Fig. 5). The response of the biosensor was examined in the presence of different interferences with a glucose concentration of 0.5 mM. In the presence of uric acid (UA, 0.5 mM) and ascorbic acid (AA, 0.1 mM), no change in the response of the biosensor was found. The result is the same as that of without interferences.

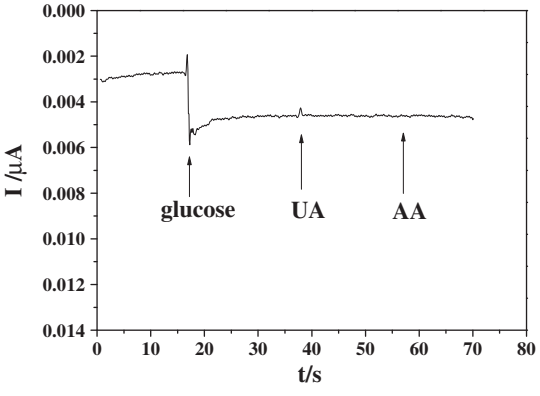


Fig. 5. Amperometric responses of the biosensor upon additions of glucose (0.5 mM), UA (0.1 mM) and AA (0.5 mM) in PBS.

Table 1
Comparison of the responses of some glucose biosensors based on different modified electrode materials.

Biosensor	Detection limit (mM)	Linear range (mM)	R-square	Ref.
Gold nanoparticle/CS/GOD	0.37	0.4–10.7	0.999	[50]
GrEC/CS-CNTs/GOD	0.016	Up to 1.0	0.999	[51]
GOD-graphene-CS/C	0.02	0.08–12	–	[52]
Graphene/Au NPs/CS/GOD	0.18	2–14	0.999	[53]
PCF-Pyr-CS/GOD/C	0.107	0.8–4	0.98	[54]
PCF-Pyr-CS/GOD/CNTs/C	0.031	0.1–1	0.998	[54]
CS–PPy	0.0155	0.5–147	0.999	This work

The performance of the proposed method was compared with other methods [50–54] as shown in Table 1. The comparative data suggest superiority of the present sensor over some earlier reported glucose biosensors. The possible reason is that PPy has a favorable electrical conductivity and CS provides a friendly microenvironment to retain GOD's bioactivity. In addition, the 3D micro-nano topological architecture of CS–PPy nanocomposites provides a significant increase in the effectiveness for GOD loading on the electrode surface, decreases the reorganizational energy for electron transfer, and allows the electroactive probe to easily diffuse through the films.

The reproducibility of the biosensors was investigated by detecting 1×10^{-3} M analyte. The relative standard deviation (RSD) for glucose was evaluated by thrice measurements to be 4.3%; and the RSD for glucose was estimated with five biosensors fabricated independently to be 5.8%. The results indicated the fabrication reproducibility of five biosensors shows an acceptable reproducibility. The stability of biosensor for glucose was examined by measuring current responses after storage in PBS at 4 °C for 7 days. The current responses of the biosensor to 1×10^{-3} M substrate did not show an obvious decline, which demonstrates that the biosensor possessed good stability.

4. Conclusion

In all, a high sensitivity glucose biosensor was assembled by immobilizing enzymes on the surface of CS–PPy nanocomposites. The CS–PPy nanocomposites could provide a biocompatible microenvironment for enzyme, and the three-dimensional micro-nano topological structure of the CS–PPy nanocomposites could enhance the electron transfer between the enzyme and electrode surface significantly. The electrode coated with the CS–PPy nanocomposites showed rapid response (5 s), broad linear range (5.00×10^{-4} to 1.47×10^{-1} M) and low detection limit (1.55×10^{-5} M). Reproducibility and the stability of the biosensor were obtained due to the high biocompatibility of the CS–PPy nanocomposites. Such novel assembly of the biosensor provided a promising platform for clinical detection in the future.

Acknowledgments

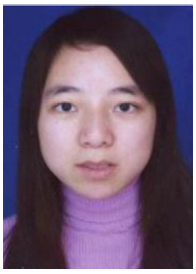
This work was supported by the National Natural Science Foundation of China (21144002), Natural Science Foundation of Jiangsu Province of China (BK2011781), RFDP (20103207120005), the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, and Base of production, education & research of prospective joint research project of Jiangsu Province (BY2011109).

References

[1] S. Cosnier, M. Holzinger, Electrosynthesized polymers for biosensing, Chem. Soc. Rev. 40 (2011) 2146–2156.
[2] M. Gerard, A. Chaubey, B.D. Malhotra, Application of conducting polymers to biosensors, Biosens. Bioelectron. 17 (2002) 345–359.
[3] N.J. Ronkainen, H.B. Halsall, W.R. Heineman, Electrochemical biosensors, Chem. Soc. Rev. 39 (2010) 1747–1763.

[4] M.J. Whitcombe, I. Chianella, L. Larcombe, S.A. Piletsky, J. Noble, R. Porter, A. Horgan, The rational development of molecularly imprinted polymer-based sensors for protein detection, Chem. Soc. Rev. 40 (2011) 1547–1571.
[5] V. Tsouti, C. Boutopoulos, I. Zergioti, S. Chatzandroulis, Capacitive microsystems for biological sensing, Biosens. Bioelectron. 27 (2011) 1–11.
[6] S. Vaddiraju, I. Tomazos, D.J. Burgess, F.C. Jain, F. Papadimitrakopoulos, Emerging synergy between nanotechnology and implantable biosensors: a review, Biosens. Bioelectron. 25 (2010) 1553–1565.
[7] X.L. Feng, L.B. Liu, S. Wang, D.B. Zhu, Water-soluble fluorescent conjugated polymers and their interactions with biomacromolecules for sensitive biosensors, Chem. Soc. Rev. 39 (2010) 2411–2419.
[8] J.D. Newman, A.P.F. Turner, Home blood glucose biosensors: a commercial perspective, Biosens. Bioelectron. 20 (2005) 2435–2453.
[9] Q. Xu, C. Mao, N.N. Liu, J.J. Zhu, J. Sheng, Immobilization of horseradish peroxidase on O-carboxymethylated chitosan/sol–gel matrix, React. Funct. Polym. 66 (2006) 863–870.
[10] J. Li, S.N. Tan, H.L. Ge, Silica sol–gel immobilized amperometric biosensor for hydrogen peroxide, Anal. Chim. Acta 335 (1996) 137–145.
[11] X.H. Chen, Y.B. Hu, G.S. Wilson, Glucose microbiosensor based on alumina sol–gel matrix/electropolymerized composite membrane, Biosens. Bioelectron. 17 (2002) 1005–1013.
[12] G. Wang, J.J. Xu, H.Y. Chen, Z.H. Lu, Amperometric hydrogen peroxide biosensor with sol–gel/chitosan network-like film as immobilization matrix, Biosens. Bioelectron. 18 (2003) 335–343.
[13] J.J. Sun, Immobilization of horseradish peroxidase on a self-assembled monolayer modified gold electrode for the detection of hydrogen peroxide, Analyst 123 (1998) 1365–1366.
[14] J.C. Vidal, E. Garca, J.R. Castillo, In situ preparation of overoxidized PPy/oPPD bilayer biosensors for the determination of glucose and cholesterol in serum, Sens. Actuators B 57 (1999) 219–226.
[15] S. Garabagiu, G. Mihailescu, Simple hemoglobin–gold nanoparticles modified electrode for the amperometric detection of acrylamide, J. Electroanal. Chem. 659 (2011) 196–200.
[16] X.L. Ren, X.W. Meng, D. Chen, F.Q. Tang, J. Jiao, Using silver nanoparticle to enhance current response of biosensor, Biosens. Bioelectron. 21 (2005) 433–437.
[17] J.S. Ye, Y. Wen, W.D. Zhang, L.M. Gan, G.Q. Xu, F.S. Sheu, Nonenzymatic glucose detection using multi-walled carbon nanotube electrodes, Electrochem. Commun. 6 (2004) 66–70.
[18] X.L. Ren, X.W. Meng, F.Q. Tang, Preparation of Ag–Au nanoparticle and its application to glucose biosensor, Sens. Actuators B 110 (2005) 358–363.
[19] X.H. Kang, Z.B. Mai, X.Y. Zou, P.X. Cai, J.Y. Mo, A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotube-modified glassy carbon electrode, Anal. Biochem. 363 (2007) 143–150.
[20] J.Y. Sun, K.J. Huang, S.F. Zhao, Y. Fan, Z.W. Wu, Direct electrochemistry and electrocatalysis of hemoglobin on chitosan-room temperature ionic liquid-TiO_2-graphene nanocomposite film modified electrode, Bioelectrochemistry 82 (2011) 125–130.
[21] L.Q. Rong, C. Yang, Q.Y. Qian, X.H. Xia, Study of the nonenzymatic glucose sensor based on highly dispersed Pt nanoparticles supported on carbon nanotubes, Talanta 72 (2007) 819–824.
[22] C.L. Bian, Q.X. Zeng, H.Y. Xiong, X.H. Zhang, S.F. Wang, Electrochemistry of norepinephrine on carbon-coated nickel magnetic nanoparticles modified electrode and analytical applications, Bioelectrochemistry 79 (2010) 1–5.
[23] S. Mojtaba, N. Mostafa, H.M.R. Milani, Highly improved electrooxidation of glucose at a nickel(II) oxide/multi-walled carbon nanotube modified glassy carbon electrode, Bioelectrochemistry 77 (2010) 120–124.
[24] R. Gill, L. Bahshi, R. Freeman, I. Willne, Optical detection of glucose and acetylcholine esterase inhibitors by H_2O_2-sensitive CdSe/ZnS quantum dots, Angew. Chem. Int. Ed. 47 (2008) 1676–1679.
[25] N.C. Foulds, C.R. Lowe, Enzyme entrapment in electrically conducting polymers. Immobilisation of glucose oxidase in polypyrrole and its application in amperometric glucose sensors, J. Chem. Soc. Faraday Trans. 182 (1986) 1259–1264.
[26] N.C. Foulds, C.R. Lowe, Immobilization of glucose oxidase in ferrocene-modified pyrrole polymers, Anal. Chem. 60 (1988) 2473–2478.
[27] C. Malitesta, F. Palmisano, L. Torsi, P.G. Zambonin, Glucose fast-response amperometric sensor based on glucose oxidase immobilized in an electropolymerized poly(o-phenylenediamine) film, Anal. Chem. 62 (1990) 2735–2740.
[28] T. Livache, A. Roget, E. Dejean, C. Barthet, G. Bidan, R. Téoule, Preparation of a DNA matrix via an electrochemically directed copolymerization of pyrrole and oligonucleotides bearing a pyrrole group, Nucleic Acids Res. 22 (1994) 2915–2921.
[29] Y.M. Uang, T.C. Chou, Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions, Biosens. Bioelectron. 19 (2003) 141–147.
[30] J. Wang, M. Musameh, Carbon-nanotubes doped polypyrrole glucose biosensor, Anal. Chim. Acta 539 (2005) 209–213.
[31] J. Baur, M. Holzinger, C. Gondran, S. Cosnier, Autoassemblage de macromolécules biologiques via des poly (pyrroles) et/ou des nanotubes de carbone fonctionnalisés, Electrochem. Commun. 12 (2010) 1287–1290.
[32] B.D. Malhotra, A. Chaubey, S.P. Singh, Prospects of conducting polymers in biosensors, Anal. Chim. Acta 578 (2006) 59–74.
[33] Y.M. Li, G.C. Li, H.R. Peng, K.Z. Chen, Facile synthesis of electroactive polypyrrole–chitosan composite nanospheres with controllable diameters, Polym. Int. 60 (2011) 647–651.
[34] Rajesh, T. Ahuja, D. Kumar, Recent progress in the development of nano-structured conducting polymers/nanocomposites for sensor applications, Sens. Actuators B 136 (2009) 275–286.
[35] B. Krajewska, Application of chitin- and chitosan-based materials for enzyme immobilizations: a review, Enzyme Microb. Technol. 35 (2004) 126–139.
[36] N.V.R.K. Majeti, A review of chitin and chitosan applications, React. Funct. Polym. 46 (2000) 1–27.
[37] H. Sashiwa, S. Aiba, Chemically modified chitin and chitosan as biomaterials, Prog. Polym. Sci. 29 (2004) 887–908.
[38] A.A. Sunil, N.M. Nadagouda, M.A. Tejraj, Recent advances on chitosan-based micro- and nanoparticles in drug delivery, J. Control. Release 100 (2004) 5–28.
[39] J. Zhou, C. Elson, T.D.G. Lee, Reduction in postoperative adhesion formation and re-formation after an abdominal operation with the use of N, O-carboxymethyl chitosan, Surgery 135 (2004) 307–312.
[40] B. Duran, D. Ak, A. Cetin, T. Guvenal, M. Cetin, A.G. Imir, Reduction of postoperative adhesions by N, O-carboxymethylchitosan and spermine NONOate in rats, Exp. Anim. 52 (2003) 267–272.
[41] L. Zheng, X.G. Chen, W.S. Liu, X.T. Han, X.J. Yan, Measurements of ψ (2S) decays into vector-tensor final states, Prog. Biochem. Biophys. 30 (2003) 314–320.
[42] L.Y. Chen, Y.M. Du, X.Q. Zeng, Relationships between the molecular structure and moisture-absorption and moisture-retention abilities of carboxymethyl chitosan: II. Effect of degree of deacetylation and carboxymethylation, Carbohydr. Res. 338 (2003) 333–340.
[43] E. Khor, J. Liang Hee Whey, Interaction of chitosan with polypyrrole in the formation of hybrid biomaterials, Carbohydr. Polym. 26 (1995) 183–187.
[44] G.H. Zhang, N.B. Yang, Y.L. Ni, J. Shen, W.B. Zhao, X.H. Huang, A H_2O_2 electrochemical biosensor based on biocompatible PNIPAM-g-P (NIPAM-co-St) nanoparticles and multi-walled carbon nanotubes modified glass carbon electrode, Sens. Actuators B 158 (2011) 130–137.
[45] Q. Xu, C. Mao, N.N. Liu, J.J. Zhu, J. Sheng, Direct electrochemistry of horseradish peroxidase based on biocompatible carboxymethyl chitosan–gold nanoparticle nanocomposite, Biosens. Bioelectron. 22 (2006) 768–773.
[46] K. Niwa, M. Furukawa, K.J.I. Niki, Ir reflectance studies of electron transfer promoters for cytochrome c on a gold electrode, J. Electroanal. Chem. 245 (1988) 275–285.
[47] G. Irace, E. Bismuto, F. Savy, G. Colonna, Unfolding pathway of myoglobin: molecular properties of intermediate forms, Arch. Biochem. Biophys. 244 (1986) 459–469.
[48] Y.L. Chen, H.B. Mao, X.F. Zhang, Y.D. Gong, N.M. Zhao, Thermal conformational changes of bovine fibrinogen by differential scanning calorimetry and circular dichroism, Int. J. Biol. Macromol. 26 (1999) 129–134.
[49] L.Q. Yang, X.L. Ren, F.Q. Tang, L. Zhang, A practical glucose biosensor based on Fe_3O_4 nanoparticles and chitosan/nafion composite film, Biosens. Bioelectron. 25 (2009) 889–895.
[50] D. Feng, F. Wang, Z.L. Chen, Electrochemical glucose sensor based on one-step construction of gold nanoparticle–chitosan composite film, Sens. Actuators B 138 (2009) 539–544.
[51] X.H. Kang, J. Wang, H. Wu, I.A. Aksay, J. Liu, Y.H. Lin, Glucose oxidase–graphene–chitosan modified electrode for direct electrochemistry and glucose sensing, Biosens. Bioelectron. 25 (2009) 901–905.
[52] M.E. Ghica, R. Pauliukaite, O. Fatibello-Filho, C.M.A. Brett, Application of functionalised carbon nanotubes immobilised into chitosan films in amperometric enzyme biosensors, Sens. Actuators B 142 (2009) 308–315.
[53] C.S. Shan, H.F. Yang, D.X. Han, Q.X. Zhang, A. Ivaska, L. Niu, Graphene/AuNPs/chitosan nanocomposites film for glucose biosensing, Biosens. Bioelectron. 25 (2010) 1070–1074.
[54] A.M. Parra-Alfambra, E. Casero, M.A. Ruiz, L. Vázquez, F. Pariente, E. Lorenzo, Carbon nanotubes/pentacyanoferrate-modified chitosan nanocomposites platforms for reagentless glucose biosensing, Anal. Bioanal. Chem. 401 (2011) 883–889.

Yi Fang received his BS degree in 2010 from Nanjing Normal University, China. Currently he is pursuing his MS degree at the same school. His current research interests include synthesis, characterization, and biosensor development based on nanomaterials.



Yalong Ni received her BS degree in 2010 from Xuzhou Normal University, China. Now she is a master in the college of chemistry and material science, Nanjing Normal University, China. Her current research interests include synthesis and characterization of biomaterials.

Guohui Zhang received her BS degree in 2008 from Nanjing Normal University, China. Currently she is pursuing her MS degree at the same school. Her current research interests include synthesis, characterization, and biosensor development based on nanomaterials.

Chun Mao received his Ph.D degree in Polymer science from Nanjing University, China in 2004. He is currently a professor at the Jiangsu Key Laboratory of Biofunctional Materials Research Group in Nanjing Normal University. His research activities focus on the preparation, characterization and application of bionanoparticles and biopolymers.

Xiaohua Huang received her BS degree in chemistry from Anhui Normal University, China in 1982 and class of post-graduates in chemistry from Peking University, China in 1987. She is presently a professor in College of Chemistry and Materials Science, Nanjing Normal University. Her current research interests include nanomaterials-based electrochemical sensors, and the microscopic chemical process of metal ions and medical nanomaterials acting on cells and enzymes.

Jian Shen received his BS in Polymer Chemistry from Nanjing University, Nanjing, China in 1982. He completed his Ph.D. in Polymer Chemistry at Nanjing University of Science and Technology, Nanjing, China in 2005. He is presently a professor (supervisor for Ph.D. student) in Research Center of Surface and Interface Chemistry and Engineering Technology, Nanjing University. His current research interests include molecule design and synthesis of the novel anticoagulant materials.