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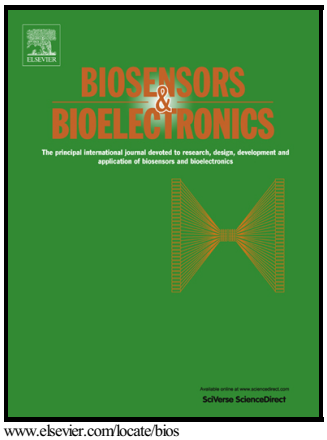

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Development of Glucose Biosensors Based on Nanostructured Graphene-Conducting Polyaniline Composite

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Abstract: A biosensor was fabricated by immobilizing glucose oxidase (GOD) into nanostructured graphene (GRA)-conducting polyaniline (PANI) nanocomposite, which was based on electrochemical polymerization of aniline in GRA synthesized by using electrochemical expansion of graphite in propylene carbonate electrolyte. Scanning electron spectroscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to characterize the morphology and performance of ~~constructed~~ the as-prepared biosensor, respectively. Amperometric measurements were carried out to optimize test conditions (pH and applied potential) of the biosensor. Under the optimal conditions, the biosensor showed a linear range from 10.0μM to 1.48mM (R^2=0.9988) with a sensitivity of 22.1μA mM^{-1} cm^{-2}, and a detection limit of 2.769μM (S/N=3). The apparent Michaelis-Menten constant (K_M^a) was estimated to be 3.26mM. The interference from glycine (Gly), D-galactose (D-Gal), urea (Urea), L-phenylalanine (L-Phe), ascorbic acid (AA), and L-tyrosine (L-Tyr) was also investigated. The results indicated that the biosensor exhibit high sensitivity and superior selectivity, providing a hopeful candidate for glucose biosensing.

Keywords: Polyaniline-graphene Graphene-polyaniline nanocomposite; Glucose oxidase; Biosensor; Selectivity

1. Introduction

Diabetes has always been a healthy problem in the world, which is due to deficiency of insulin and hyperglycemia, or beta cell dysfunction of pancreas, and is manifested as blood glucose concentration higher than normal range of 4.4~6.6mM

(Canivell and Gomis, 2014; Wang, 2008). Therefore, it's of great significance to determine and monitor blood glucose concentration in a sensitive, fast and accurate way.

Since Clark and Lyons first initiated the concept of glucose biosensor in 1962 (Clark and Lyons, 1962), numerous approaches have been developed for glucose detection, involving colorimetric method (Zhang et al., 2014), flow injection analysis (Wang and Huang, 2003), electrochemiluminescence (Tian et al., 2014), quantum dots (Lv et al., 2014), etc. Majority of these methods are based on the detection of hydrogen peroxide which is produced by enzyme catalyzed reaction between GOD and glucose (Yang et al., 2014). Among all these means, electrochemical biosensors (Kong et al., 2014; Shervedani et al., 2006; Wang et al., 2013), because of their simplicity of fabrication process and application, low cost and excellent performance, are one of the most popular methods, and thus have been a hotspot in this field during the past several decades. Till date, electrochemical glucose biosensors have roughly experienced three generations (Wang, 2001, 2008). For the first- and second-generation glucose biosensors, errors from oxygen deficiency, interfering species, or the competitive reactions of oxygen and artificial mediator with reduced state of GOD, may affect the accuracy of glucose determination. Because GOD active center, flavin adenine dinucleotide (FAD), is enwrapped with a thick protein layer, and thus form an inherent barrier for direct electron transfer , another problem affecting the performance of glucose biosensors is how to improve the electron transfer between FAD and electrode surface (Liang et al., 2013; Wang, 2008). To

solve these problems, the point is to develop more suitable functional materials as matrix to improve sensitivity or selectivity of biosensors. Therefore, large number of materials have been applied to glucose biosensors, like cellulose acetate (Ren et al., 2009), polyacrylonitrile (Zheng et al., 2002), conductive polymers, like polypyrrole (Chen et al., 2006), especially PANI (Dhand et al., 2010) and poly (phenylendiamine) (Garjonyte and Malinauskas, 1999), carbon materials such as carbon nanotubes (CNTs) (Zhu et al., 2012) and GRA (Unnikrishnan et al., 2013), etc.

PANI is a popular conducting polymer with good chemical stability (Marcasuzaa et al., 2010), high conductivity, and low operational potential (Devi et al., 2013), and has been widely applied to glucose biosensors. Gvozdenović et al. (2011) studied a glucose biosensor based on PANI/GOD with a linear range of 1.0-20mM and K_M^a of 0.30mM. On the other hand, GRA, which is composed of a two-dimensional, mono-layered nanosheet that is constituted of sp^2-hybridized carbon atoms densely packed in a honeycomb crystal-lattice (Chaturvedi et al., 2014; Yang et al., 2013), is a rising star owing to its superior performance, like mechanical strength (Ovid'ko, 2013), excellently high conductivity (Devi et al., 2013) and superior mobility of charge carriers (Chaturvedi et al., 2014). Therefore, GRA has been widely used in electrochemical biosensing, such as glucose determination and single nucleotide polymorphisms detection for DNA (Akhavan et al., 2012), early disease diagnose (Akhavan et al., 2014) and other fields. Unnikrishnan et al. (2013) fabricated a GRA-GOD glucose biosensor with a linear range of 0.1-27mM (R^2=0.988) and a sensitivity of 1.85μA mM^{-1} cm^{-2}. Kang et al. (2009) constructed a glucose biosensor

based on GOD-GRA-chitosan (CS) composite which exhibited a linear range of 0.08-12mM (R^2=0.9993) with a sensitivity of 37.93μA mM^{-1} cm^{-2} and a detection limit of 20μM. The two materials can be compounded by physical (like blending) or chemical (like grafting) methods to form a superior composite for glucose immobilization due to their respective advantages and synergistic effects.

Although there are many researches performed on immobilization of GOD into PANI or GRA, few studies were carried out by using nanocomposite containing these two materials as a matrix for GOD immobilization, such as GRA/PANI/Gold nanoparticles (Au NPs) composite (Kong et al., 2014; Xu et al., 2014). In this study, based on the previous work of our group (Zheng et al., 2002), a novel electrochemical method was used to construct a glucose biosensor by immobilizing GOD into GRA-PANI nanocomposite, which was synthesized through electrochemical polymerization of ANI aniline in GRA. The nanostructured GRA, prepared by means of electrochemical expansion of graphite in propylene carbonate electrolyte, showed excellent electrical conductivity. Moreover, scanning electron microscopy (SEM) was used to characterize the morphology of the biosensor. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were adopted to examine the performance of the biosensor. Amperometric measurements, selectivity, stability and practical application to human plasma of the biosensor were also studied.

2. Material and methods

2.1. Reagents and materials

Glucose oxidase (GOD, BC grade) from Aspergillus niger with an activity of

100U mg^{-1} was purchased from Sigma. D-galactose, glycine, L-tyrosine, L-phenylalanine, urea, aniline and chitosan (CS, deacetylation≥95%) were obtained from Aladdin. Glucose, ascorbic acid, sodium phosphate monobasic dihydrate and sodium phosphate dibasic dodecahydrate were bought from Sinopharm Chemical Reagent Co., Ltd. All other reagents and chemicals were of analytical grade and were used as received without further purification.

2.2. Instrumental

The cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and amperometric measurements were performed using a PARSTAT 4000 electrochemical workstation (AMETEK, USA). A typical three-electrode system was used in this work with saturated calomel electrode (SCE) as the reference electrode, platinum (Pt) disk electrode with a diameter of 2mm as the counter electrode and modified Pt disk electrode as the working electrode. Scanning electron microscopic images were obtained by SU1510 scanning electron spectroscopy (SEM) (Hitachi).

2.3. Preparation of GRA-PANI modified electrodes

Before the fabrication of the modified electrodes, the treatment of bare Pt disk electrodes was required. Pt disk electrodes were polished with 1.5μm, 0.5μm, and 50 nm alumina slurries, respectively. And then the electrodes were sonicated with ultra-pure water, ethanol, and ultra-pure water successively. After sonication, the bare Pt disk electrodes were treated by CV with a potential window of -0.2~ 1.6V (vs. SCE) at a scan rate of 0.2V/s in 0.2M sulfuric acid (H_2SO_4) until stable cyclic voltammmogramms (CVs) were achieved. Finally, the electrodes were rinsed with

ultra-pure water and dried with filter paper.

GRA was synthesized by electrochemical expansion of graphite in propylene carbonate electrolyte according to the literature (Wang et al., 2011). The GRA-PANI modified electrodes were fabricated by cyclic voltammetry. The details were as follows: 5mg GRA was weighed in a 25mL beaker, and 10 mL 1M hydrochloride (HCl) solution was added to the beaker; then this mixed solution was sonicated for about 1h to make GRA dispersing in the HCl solution uniformly; finally, 182μL aniline was added to the aforesaid solution and stirred fully to obtain the electrolyte solution. GRA-PANI nanocomposite was obtained on the surface of the Pt disk electrode using electrochemical polymerization of aniline in GRA/HCl mixed solution with a potential window of -0.1~ 1V (vs. SCE) at a scan rate of 20mV/s.

2.4. Fabrication of the amperometric glucose biosensors

CS-GOD solution was prepared by mixing 8mg/mL GOD in 0.02M phosphate buffer (pH 7.0) and 0.5wt% CS/acetic acid (HAc) at a volume ratio of 1:1. The GRA-PANI/CS-GOD modified electrode was fabricated by coating 5μL CS-GOD solution onto the surface of GRA-PANI and transforming into bio-nanocomposite membrane through phase inversion process (Zheng et al., 2002). The glucose biosensor was preserved in 0.02M phosphate buffer (pH 7.0) and stored in a 4℃ refrigerator. The PANI/CS-GOD biosensor was constructed according to the same process without the presence of GRA.

2.5. Electrochemical measurements

All electrochemical measurements were carried out in a three-electrode

electrochemical cell at room temperature of 25℃. CVs were obtained with a potential window of 0.2~ 0.9V (vs. SCE) at a scan rate of 50mV/s in 0.02M phosphate buffer (pH 7.0). EIS measurements were performed in 0.02M phosphate buffer (pH 7.0) with the amplitude of 10 mV and frequencies ranging from 100 kHz to 0.1Hz. All amperometric measurements were carried out at an applied potential of 0.65V (vs. SCE) in 0.02M phosphate buffer (pH 7.0) without specific description, requiring the transient background to decrease to a steady-state value. A magnetic stirring was applied to the solution during amperometric measurements to achieve convective mass transfer.

3. Results and discussion

3.1. Morphology characterization of the biosensors

Scanning electron microscopic (SEM) images of (A) GRA, (B) PANI, (C) GRA-PANI and (D) GRA-PANI/CS-GOD were shown in Fig.1. From Fig. 1A, it was found that GRA exhibited a curved and flake-like structure. Moreover, according to the Raman spectra, GRA showed monolayer or few-layer sheet structure constituted of carbon atoms consistent with the results of literature (Wang et al., 2011), which endows GRA with impermeability in molecular level (Berry, 2013; Novoselov et al., 2012). Therefore, GRA can be used as an excellent physical isolation layer. PANI presented a rod-like shape, with a diameter of about 300nm and a length of some 1.5μm, and aggregated together, forming a coral-like structure (Fig. 1B). When PANI and GRA were composited by electrochemical method, the nanocomposite showed a larger diameter but a shorter length (Fig. 1C) compared to pure PANI, which was a

result of the preparation method of the nanocomposite. PANI was deposited onto the surface of Pt disk electrode by the electrochemical polymerization of aniline, while GRA was absorbed physically onto the electrodes with the deposition of PANI. Moreover, the concentration of GRA (0.5mg/mL) was much smaller than that of aniline (18.626mg/mL) in the electrolyte solution, and thus the nanocomposite was composed of majority of PANI and small amount of GRA. Therefore, it was inferred that GRA was enwrapped by PANI to form the nanocomposite. The SEM image of the nanocomposite with higher magnification (Fig. S1) further indicated that small amount of GRA be existed in the GRA-PANI nanocomposite. After GOD immobilization, GRA-PANI/CS-GOD (Fig. 1D) exhibited a three-dimensional network structure with CS as a linker, and GOD embedded into the nanocomposite.

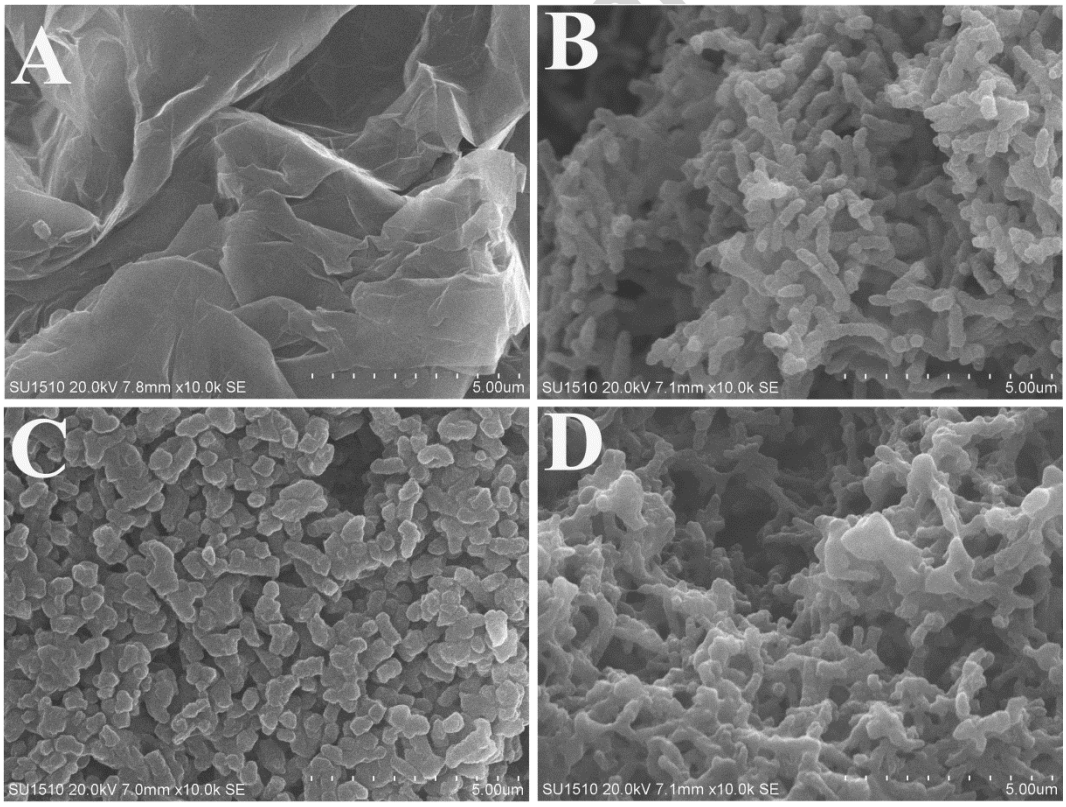

Fig. 1. SEM images of (A) GRA, (B) PANI, (C) GRA-PANI nanocomposite and (D) GRA-PANI/CS-GOD.

3.2. Cyclic voltmmetry and electrochemical impedance spectroscopy of the biosensors

The cyclic voltammograms (CVs) of different modified electrodes were given in Fig. 2. No obvious differences of current response between the absence and presence of 0.598mM glucose were observed for PANI (curve a and curve b), PANI/CS (curve c and curve d) and GRA-PANI/CS (curve e and curve f) modified electrodes (Fig. 2A), which suggested that PANI, CS and GRA-PANI nanocomposite have no catalytic effects on the reaction of glucose. However, for PANI/CS-GOD and GRA-PANI/CS-GOD modified electrodes in Fig. 2B, the oxidation currents for both electrodes increased obviously after adding glucose (curve b and curve d) when compared with their respective CVs without glucose (curve a and curve c). This indicated that GOD played a significant role in the reaction of glucose. Fig. 2B also revealed that the oxidation current of GRA-PANI/CS-GOD modified electrode (curve d) was much higher than that of PANI/CS-GOD modified electrode (curve b), which was owing to excellent conductivity and electron transfer of GRA and its synergistic effect with PANI, resulting in amplification of the oxidation current. The reaction mechanism could be interpreted as the following two steps: (1) glucose is catalyzed to gluconic acid and hydrogen peroxide with GOD in the presence of oxygen; (2) oxidation of hydrogen peroxide occurs at a positive applied potential, and the oxidation current is detected by the glucose biosensor, which could be described as follows (Wang, 2008; Wang et al., 2013).

$$glucose + O_2 \xrightarrow{GOD} gluconic\ acid + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

The electrochemical impedance spectroscopy (EIS) of different modified electrodes was seen in Fig. 2C. The Nyquist plot of electrochemical impedance spectra consists of two sections: a semicircle part in high frequencies which reflects electron transfer limited process at electrode surface and a linear part in low frequencies corresponding to diffusion limited process. And the electron transfer resistance R_{ct} can be fitted as the semicircle diameter. It was obvious that R_{ct} of GRA-PANI (5901Ω, curve a), GRA-PANI/CS (56432Ω, curve b) and GRA-PANI/CS-GOD (64546Ω, curve c) modified electrodes were quiet small and even negligible when compared to those of PANI (285320Ω, curve d), PANI/CS (1060800Ω, curve e) and PANI/CS-GOD (1434600Ω, curve f) modified electrodes, suggesting that the conductivity of GRA-PANI nanocomposite be much better than that of PANI, which ~~is~~ was a result of the super high electric conductivity of GRA and its synergistic effects with PANI, and thus more suitable for GOD-based biosensors. On the other hand, after GOD was immobilized into GRA-PANI nanocomposite (or PANI) matrix, the corresponding R_{ct} increased slightly while the corresponding slops somewhat decreased, indicating that GOD was well immobilized into the matrix, leading to a slight hindrance in both electron transfer process and diffusion process.

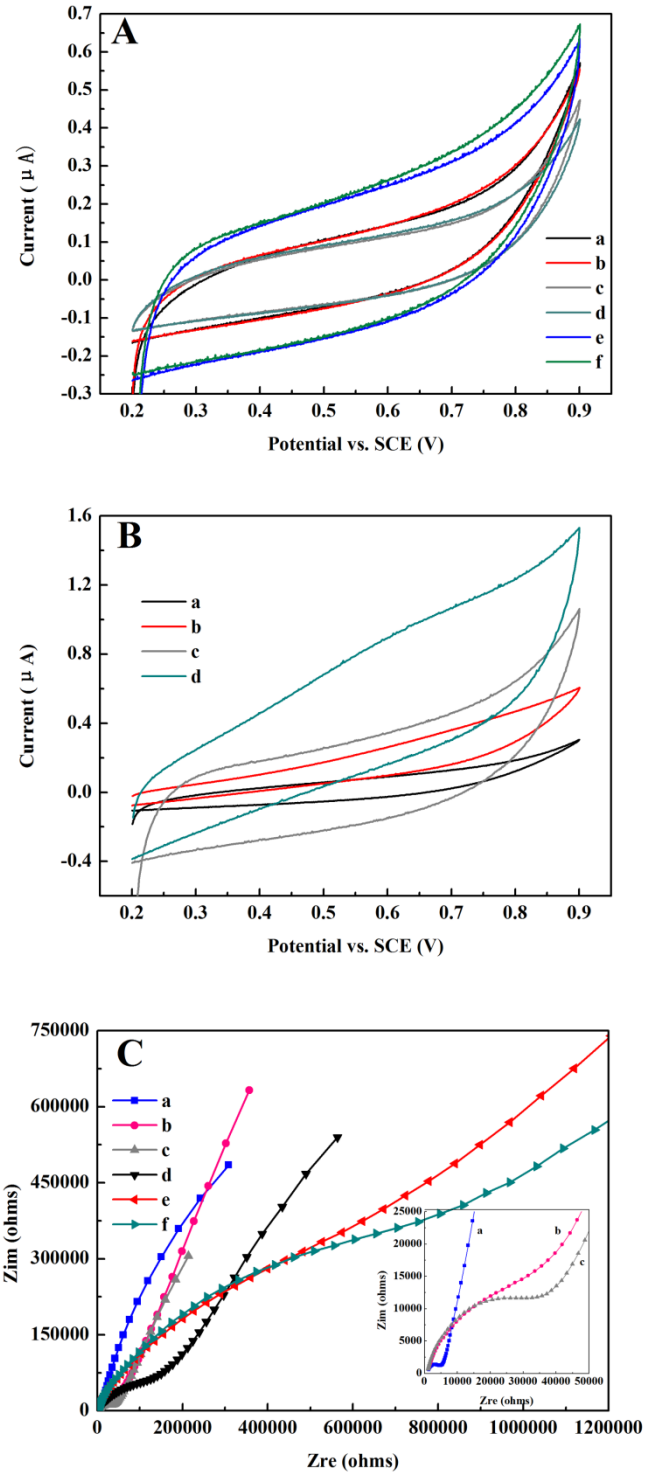

Fig. 2. (A) CVs in 0.02M phosphate buffer (pH 7.0) at 50mV/s for (a) without glucose, and (b) with 0.598mM glucose of PANI modified electrode, (c) without glucose, and (d) with 0.598mM glucose of PANI/CS modified electrode, (e) without glucose, and (f) with 0.598mM glucose of GRA-PANI/CS modified electrode. (B) CVs in 0.02M phosphate buffer (pH 7.0) at 50mV/s for (a) without glucose, and (b) with 0.598mM glucose of PANI/CS-GOD modified electrode, (c) without glucose, and (d) with 0.598mM glucose of GRA-PANI/CS-GOD modified electrode. (C) EIS of different modified Pt disk electrodes in 0.02M phosphate buffer (pH 7.0) for (a) GRA-PANI, (b) GRA-PANI/CS, (c) GRA-PANI/CS-GOD, (d) PANI, (e) PANI/CS and (f) PANI/CS-GOD modified electrodes (The inset

figure showed the EIS for (a) GRA-PANI, (b) GRA-PANI/CS and (c) GRA-PANI/CS-GOD modified electrodes).

3.3. Optimal conditions of the biosensors

Applied potential and pH values of the buffers were two important factors which have influence on the current response of the constructed biosensor. As was shown in Fig. 3A, the oxidation current response of the biosensor increased gradually with applied potential increasing (0.3V~0.65V (vs. SCE)), reaching a maximum value at 0.65V (vs. SCE), and then decreased with the applied potential increasing continuously (0.65V~0.8V (vs. SCE)). Therefore, 0.65V (vs. SCE) was chosen as the optimal applied potential in the subsequent measurements.

Another important factor that influences the current response of the glucose biosensor is pH value of the buffer which was used as a background solution of glucose. It was obvious that the influence of pH on the current response was relatively smaller than that of applied potential. The oxidation current of the glucose biosensor increased with the increase of pH value (pH 3.5~6.0), then reached a relatively stable level at pH 6.0~8.0, and finally decreased with the subsequent increased pH (Fig. 3B). So there should be an optimal pH range from 6.0 to 8.0 where the glucose biosensor performed well, which was because that GRA-PANI nanocomposite isolated GOD from buffer solution to some extent. Therefore, the biosensor was not that sensitive to the change of pH, and a middle pH value of 7.0 as optimal pH value was available. On the above basis, the subsequent measurements were carried out in 0.02M phosphate buffer (pH 7.0) at an applied potential of 0.65V (vs. SCE).

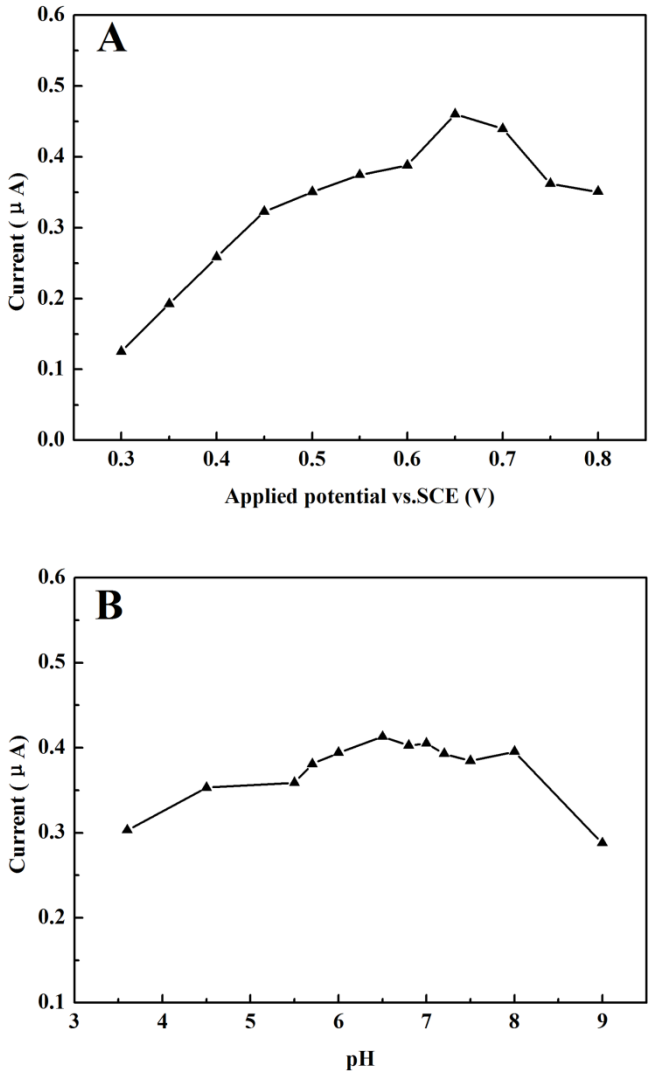

Fig. 3. Effects of (A) applied potential in 0.02M phosphate buffer (pH 7.0) containing 0.598mM glucose and (B) pH values in different buffers containing 0.598mM glucose on the current responses of GRA-PANI/CS-GOD biosensor.

3.4. Amperometric measurements of the GRA-PANI/CS-GOD biosensor

The amperometric responses of the constructed biosensors with presence and absence of GRA (Fig. 4A) were obtained by successively injecting 20.0mM and 200mM glucose respectively at an applied potential of 0.65V (vs. SCE) in 0.02M phosphate buffer (pH 7.0). It was observed from Fig. 4A that both biosensors showed amperometric responses to injection of glucose and reached 98.3% of its steady state

currents within 5s, but the biosensor with presence of GRA exhibited higher response to the same concentration of glucose, which could be contributed to superior conductivity and electron transfer ability of GRA and its synergistic effects with PANI.

From the Current-Concentration curves (Fig. 4B) for both biosensors, we could get inspiration to the reaction mechanism of glucose: In low-concentration region, the reaction of glucose follows first order reaction, that is, the current response is proportional to substrate concentration. While in relative high-concentration region, the reaction follows zero order reaction kinetics, which means the current response reaches a plateau and will not change with the further increase of glucose concentration. These are the typical characters of enzymatic reactions. When comparing curve a and curve b in Fig. 4B, it was found that the current responses reached a plateau when the glucose concentration increased to about 4.5mM for GRA-PANI/CS-GOD biosensor, while this glucose concentration was about 1.5mM for PANI/CS-GOD biosensor, which implied that the former had a much wider linear range than that of the latter.

We could gain further insight from Fig. 4C and Fig. 4D. The linear range of GRA-PANI/CS-GOD biosensor, which was wider than some other reported glucose biosensors (Marand et al., 2014; Wang et al., 2013; Xu et al., 2014), was from 10.0μM to 1.48mM (R^2=0.9988) with a higher sensitivity of 22.1μA mM^{-1} cm^{-2} compared with some published researches (Unnikrishnan et al., 2013; Wan et al., 2010). The detection limit, evaluated to be 2.769μM (S/N=3), was lower than some

already reported glucose biosensors (Hui et al., 2013; Wan et al., 2010; Wu et al., 2010). According to the Lineweaver-Burk form of Michaelis-Menten Equation: $1/I = 1/I_{Max} + K_M^a/(I_{Max}C)$, where I is steady-state current after adding a substrate, I_{Max} is the maximum current when substrate is saturated, C is the substrate concentration, and K_M^a is the apparent Michaelis-Menten constant which is independent on enzyme concentration (English et al., 2005; Xu et al., 2010). So the K_M^a was estimated to be 3.26mM (curve a in Fig. 4D). For PANI/CS-GOD biosensor, the linear range was 10.0μM to 1.09mM (R^2=0.9990) with a sensitivity of 15.7μA mM^{-1} cm^{-2} (curve b in Fig. 4C), and K_M^a was about 1.69mM (curve b in Fig. 4D). In a word, the GRA-PANI/CS-GOD biosensor performed better (like wider linear range, fast response and high sensitivity) than the PANI/CS-GOD biosensor, and some behaviors of the biosensor were more excellent than that of many other reported glucose biosensors as previously mentioned, which made it a promising candidate for glucose determination.

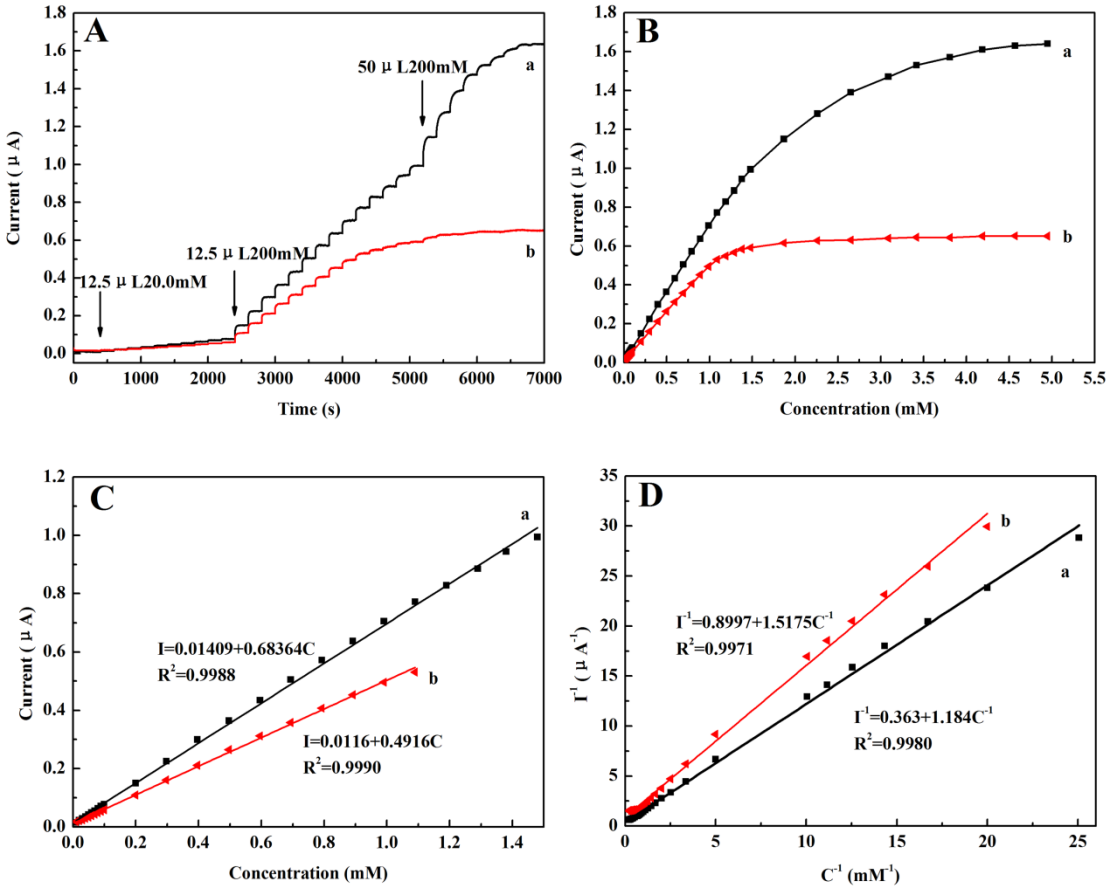

Fig. 4. (A) Amperometric responses to successive injection of glucose at an applied potential of 0.65V (vs. SCE) in 0.02M phosphate buffer (pH 7.0), (B) Current-Concentration curves, (C) Calibration curves, and (D) Calculation of apparent Michaelis-Menten constants according to the data in (B) for (a) GRA-PANI/CS-GOD and (b) PANI/CS-GOD biosensors.

3.5. Selectivity and stability of the biosensor

Influence from six endogenous interfering species (glycine (Gly), D-galactose (D-Gal), urea (Urea), L-phenylalanine (L-Phe), ascorbic acid (AA), and L-tyrosine (L-Tyr)) in human plasma on amperometric response of the biosensor was also discussed. It was documented that these endogenous species are often electroactive in positive potential region (Wilson and Ammam, 2007). For all that, the biosensor showed well-defined amperometric response to successive addition of glucose, but no response occurred to glycine, D-galactose, Urea, L-phenylalanine, and ascorbic acid,

whose concentrations were 0.199mM except L-phenylalanine (0.0990mM), and slight, even negligible response to 4.916μM L-tyrosine, which should be contributed to the excellent physical isolation of GRA and its synergistic effect with PANI (Fig. 5). Therefore, the biosensor presented superior high selectivity to the six interfering species and could be employed to determine glucose concentration in plasma samples. In addition, the selectivity of the biosensors was much higher than that of some previously reported graphene-based glucose biosensors (Choi et al., 2011; Unnikrishnan et al., 2013; Yang et al., 2013). Comparison of the behaviors of the fabricated glucose biosensor with other previous GRA-based glucose biosensors was summarized as Table S1.

The repeatability of the fabricated biosensor was measured by calculating relative standard deviations (RSD) of the biosensor response to 0.399mM and 0.698mM glucose for ten times, respectively. The RSD for 0.399mM glucose was 3.01%, and for 0.698mM glucose it was 0.75%. The reproducibility of the biosensor was evaluated by the RSD of the amperometric responses of four biosensors fabricated at different times according to the same method, to 0.598mM glucose, and the value of RSD was 4.40%. All these confirmed that the fabricated biosensor exhibited good stability.

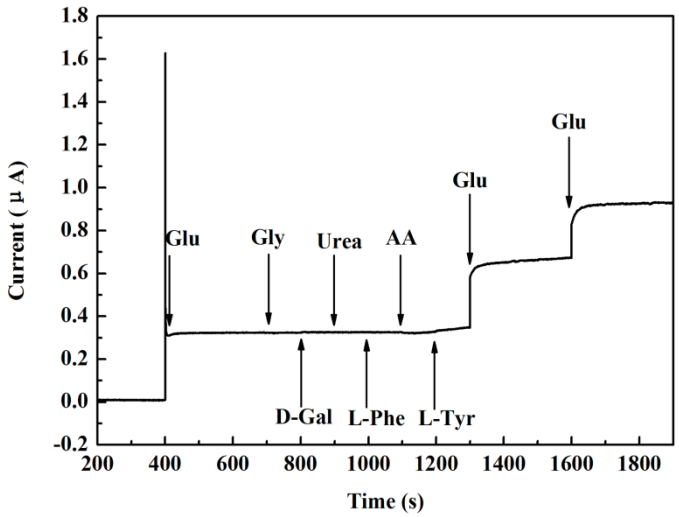

Fig. 5. Effects of the interferences on the amperometric response of the glucose biosensor in the presence of glucose.

3.6. Determination of glucose in plasma samples

To illustrate the practical application of the biosensor, the determination of glucose in human plasma samples by the biosensor was also performed. Fresh plasma samples were first analyzed in the hospital with Hitachi 7600 Model biochemical analyzer and the results were summarized in Table 1. The samples were diluted about 250 times in 0.02M phosphate buffer (pH 7.0) when measuring them by the biosensor. The resulted glucose concentrations in the plasma samples according to the linear regression equation and conversion were also listed in Table 1. The recoveries of the glucose concentration ranged from 95.6% to 104.9%. These results suggested that the glucose biosensor be used to determine glucose in human plasma samples.

Table 1

Determination of glucose in plasma samples.

Sample Number	Determined by hospital (mM)	Determined by the sensor (mM)	Recovery (%)
1	5.64	5.39	95.6
2	6.61	6.52	98.6
3	8.19	8.59	104.9
4	9.90	10.11	102.1
5	12.71	12.66	99.6
6	16.99	17.13	100.8

4. Conclusions

A glucose biosensor based on GRA-PANI/CS-GOD modified electrode fabricated by electrochemical polymerization has been developed. The constructed biosensor exhibited excellent selectivity and wide linear range from 10.0μM to 1.48mM due to the physical isolation and superior conductivity of GRA and its synergistic effect with PANI. Meanwhile, the biosensor showed good repeatability and reproducibility. The biosensor could be used to determine glucose concentration in human plasma, providing a promising candidate for glucose biosensing. The nanocomposite membrane of GRA and PANI by electrochemical polymerization is hopeful as an excellent matrix for GOD-based glucose biosensors. The new composition method and nanostructured material might be exploited for other enzyme biosensors.

Acknowledgments

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Research highlights

1. A novel glucose biosensor based on nanostructured graphene-conducting polyaniline was fabricated.

2. The graphene-polyaniline composite was synthesized by electrochemical polymerization of aniline in aniline/graphene/hydrochloride electrolyte solution.

3. Graphene, which was prepared by electrochemical expansion, displayed excellent electric conductivity.

4. Phase inversion was applied for fabrication of the biosensor.

5. The biosensor had an amplification of current response and thus showed high sensitivity.

6. The biosensor exhibited excellent selectivity at a positive applied potential.