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Improved Sensitivity and Selectivity Glucose Biosensor Based on PANI-GRA Nanocomposite Film Decorated with Pt Nanoparticles

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A novel glucose biosensor was prepared by in situ electropolymerization of aniline in GRA synthesized by using electrochemical expansion of graphite in propylene carbonate electrolyte onto platinum (Pt) electrode, followed by electrodeposition of platinum nanoparticles (PtNPs) on Pt/PANI-GRA electrode. Glucose oxidase (GOD) was immobilized on this modified Pt electrode through chitosan. The fabricated process and the electrochemical behaviors of resulting biosensor were investigated by scanning electron microscopy (SEM) and cyclic voltammetry (CV). The developed glucose biosensor exhibited superior sensitivity of 52.67 μA mM^{-1} cm^{-2} and selectivity, which showed no response to the interference from glycine (Gly), Urea (Urea), L-phenylalanine (L-Phe), ascorbic acid (AA), tyrosine (L-Tyr) and D-galactose (D-Gal). The biosensor also showed a fast response time within 3 s. The biosensor was used for the detection of glucose in human blood samples with satisfactory results compared with standard hospital laboratory analysis.

Keywords: Polyaniline; Graphene; Biosensor; Sensitivity; Selectivity

1. INTRODUCTION

Biosensors have received much attention since Clark and Lyons [1] proposed the glucose electrode based on the measurement of the oxygen consumed through the enzyme-catalyzed reaction in 1962. Substantial efforts have been mainly focused on enzyme-based biosensor for qualitative and quantitative analysis of glucose [2]. In order to facilitate their practical application such as medical diagnosis and environmental detection, especially on-line monitoring [3,4], glucose biosensors have to

meet several requirements, including sensitivity, selectivity, rapid response time and low cost. However, both superior sensitive and selective glucose biosensor still remains challenging because efficient synthesis of nanomaterial or nanocomposite is hardly achieved. The current signal can be improved by selecting a suitable matrix to accelerate the electron transfer from the active sites of enzyme to the electrode. Due to the large surface area and high conductivity of the matrix material, it contributes to increasing sensitivity. On the other hand, permselective nanomaterials can effectively reject electroactive interferents, leading to high selectivity of the biosensor. Therefore, the innumerable versatile nanomaterials have been explored for glucose biosensors including conducting polymers [5–7], mental nanoparticles [8–10], carbon nanomaterials [11], and their nanocomposites [12–14], especially polyaniline (PANI) [15] and graphene (GRA) [16].

Among various conducting polymers, PANI with long π-conjugated backbone is considered to be an attractive homopolymer owing to its excellent conductivity, good electrochemical stability and ease of preparation, which can provide moderate environment for immobilization of biomolecule, retainment of enzyme activity and amplification of signal in enzymatic reaction [17,18]. However, these conventional PANI-based biosensors still exist some disadvantages such as unsatisfied sensitivity and selectivity [18]. To improve the performance of biosensors, various methods were developed to modify PANI with nanomaterials such as metal nanoparticles and nanostructured carbon materials. Zhai [19] fabricated a PANI/PtNPs glucose biosensor by chemical preparation method with excellent sensitivity as high as 96.1 μA mM^{-1} cm^{-2} due to the highly active catalysts of PtNPs. Zhong [20] successfully synthesized multi-wall carbon nanotube (MWNT)-PANI nanocomposite film by polymerization reaction with potassium persulfate as oxidant, and then platinum nanoparticles were electrodeposited onto the surface of film. The resulting biosensor exhibited a short response time (within 5 s) and high sensitivity (16.1 μA mM^{-1}). PANI decorated with metal nanoparticles and carbon nanotube respectively improved the sensitivity of the biosensor compared with pure PANI, while the selectivity had almost no significant improvement.

As we all know, graphene is a two-dimensional structure material that consists of carbon atoms placed in a honeycomb crystal lattice bonded by sp^2 bonds [21]. The unique fascinating properties of graphene such as large surface area, excellent electrical conductivity and good mechanical strength make it an ideal candidate for the development of sensitive and selective biosensor [22–24]. Various methods and techniques have been employed to prepare versatile graphene structure, including exfoliation of graphite and reduction of graphene oxide [25]. The challenge is to retain electrical conductivity of graphene without collateral damage because of the inevitable disruption of conjugated bond in the preparation process. Wang [26] developed a mild electrochemical method to efficiently exfoliate graphite to form few-layer graphene flakes without using any oxidation process or super-strong acid. The few-layer graphene was previous used by our group as a support matrix to incorporate with PANI for glucose biosensor and the fabricated biosensor exhibited high conductivity and selectivity [27]. The glucose biosensor based on PANI-GRA-AuNPs was also developed [28], in which PANI-GRA was synthesized by stirring aniline and graphene with ammonium persulfate as initiator then immersed into the AuNPs colloid obtained by classical reduction method. The biosensor displayed a linear range from 4.0 μM to 1.12 mM, a low detection limit of 0.6 μM at signal-to-noise of 3 and good selectivity. In addition, Kong [29] proposed a paper-based sensing device modified with

PANI-GRA-AuNPs nanocomposite which was prepared by the same method mentioned above [28]. The paper disk biosensor exhibited sensitivity of 20.32 μA mM^{-1} cm^{-2} and good selectivity. These nanocomposites showed enhanced electrocatalytic activity and sensitivity compared with pure PANI, graphene and mental nanoparticles. The methods used for preparing composites, especially nanocomposites influence their nanostructure and electrochemical performance of biosensor. Comparing with typical chemical polymerization, electrochemical polymerization and electrodeposition have prominent advantages such as simplicity, controllability and reproducibility. Moreover, conducting polymer and metal nanoparticles can be fully deposited on the surface of electrode [30,31].

In order to magnify their unique properties, there are many researches performed on two or three components combined of PANI, carbon materials and mental nanoparticles, but challenges still exist in obtaining both sensitive and selective glucose biosensor that offers a great deal of promises in fulfilling applications. In this paper, based on our previous work [27,32,33], an amperometric glucose biosensor with superior sensitivity and selectivity using PANI-GRA/PtNPs nanocomposite as matrix was successfully prepared by simple two-step electrochemical deposition method (Figure. 1). Two-dimensional (2D) graphene based on exfoliation of graphite by electrochemical method could effectively combine with PANI and provided a favorable microenvironment for the following PtNPs loading, contributing to prominent electrical, physiochemical and structural properties. The PANI-GRA/PtNPs modified electrode further improved the sensitivity of the biosensor and the current signal was two times higher than that of previously reported biosensor by our group [27]. The morphology of as-synthesized nanomaterials was observed by scanning electron microscopy (SEM).

2. MATERIAL AND METHODS

2.1 Reagents and materials

GRA was synthesized by electrochemical expansion of graphite in propylene carbonate electrolyte according to the literature [26]. Glucose oxidase (GOD) (BC grade, 100 U/mg, from Aspergillus niger) was obtained from Sigma. D-galactose, glycine, L-tyrosine, L-phenylalanine, urea, aniline and chitosan (CS, deacetylation ≥95%) were purchased from Aladdin. Other chemicals were of analytical reagent grade, and all aqueous solutions were prepared with Millipore water. The collected serum samples were used within 3 h without any pretreatment for the electrochemical assays. The samples were also subject to the clinical laboratory test in parallel.

2.2 Instrumental

Electrochemical measurements were performed on a PARSTAT 4000 electrochemical workstation (AMETEK, USA), using a typical three-electrode system with saturated calomel electrode (SCE) as the reference electrode, platinum (Pt) disk electrode (2 mm in diameter) as the counter

electrode and modified Pt disk electrode as the working electrode. Scanning electron microscopic images were obtained by SU8010 scanning electron spectroscopy (SEM) (Hitachi).

2.3 Preparation of PANI-GRA/PtNPs nanocomposite

Prior to electrodeposition, Pt disk electrodes were carefully polished with 1.5 μm, 0.5 μm and 50 nm alumina slurries, followed by sonication in Millipore water, ethanol and Millipore water successively. Then the bare Pt disk electrodes were cleaned by cyclic voltammogram (CV) method with a potential range from -0.2 V to 1.6 V (vs. SCE) at a scan rate of 0.2 V/s in 0.2 M sulfuric acid (H_2SO_4). The PANI-GRA nanocomposite was initially prepared through electrochemical polymerization of 182 μL of aniline in GRA/HCl mixed solution (5 mg GRA was added in 10 mL of 0.1 M HCl) by applying 6 cycles from 0.1 V to 1 V (vs. SCE) at a scan rate of 20 mV/s.

The three-component PANI-GRA/PtNPs nanocomposite was obtained by further electrodeposition. The as-prepared PANI-GRA modified electrode was immersed into the 0.5 M H_2SO_4 electrolyte contained 3 mM H_2PtCl_6 and the electrochemical deposition process was carried out by cyclic voltammetry scanning (CV) from 0.25 V to 0.1 V (vs. SCE) at a scan rate of 20 mV/s. The resulting PANI-GRA/PtNPs nanocomposite was rinsed with Millipore water to remove residual H_2PtCl_6.

2.4 Fabrication of the biosensors

CS-GOD solution was obtained by mixing 8 mg/mL GOD with 0.02 M phosphate buffer (pH 7.0) and 0.5 wt% CS/acetic acid (HAc) at a volume ratio of 1:1. The PANI-GRA/PtNPs/CS-GOD modified electrode was fabricated by coating 5 μL CS-GOD solution onto the surface of PANI-GRA/PtNPs and the coating was dried at room temperature. The fabricated procedure of the modified electrode was demonstrated in Figure. 1. Moreover, the PANI/PtNPs/CS-GOD electrode was constructed according to the same process without GRA.

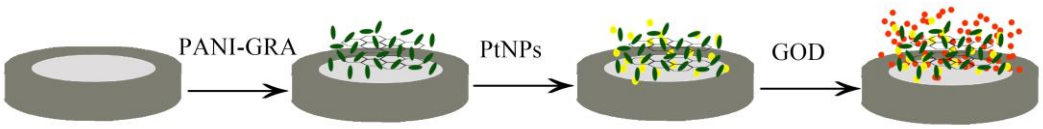

Figure 1. Schematic of the fabrication of PANI-GRA/PtNPs/CS-GOD glucose biosensor.

2.5 Electrochemical measurements

All measurements were conducted in 0.02 M phosphate buffer (pH 6.5) without specific description. The electrochemical behaviors of PANI/PtNPs and PANI-GRA/PtNPs modified electrode were investigated by cyclic voltammogram (CV) with a potential range of 0.2 V-0.9 V (vs.SCE) at a

scan rate of 50 mV/s. Amperometric measurements were performed at an applied potential of 0.55 V (vs. SCE) with a magnetic stirring at 1200 rpm. After the background current decreased at a steady-state value, small aliquots glucose solution (12.5 μL of 20 mM, 12.5 μL of 200 mM and 37.5 μL of 200 mM glucose) were successively injected every 200 s at an applied potential of 0.55 V (vs. SCE). The glucose of human plasma sample was analyzed in hospital with BECKMAN-COULTER AU5800 biochemical analyzer using hexokinase method.

3. RESULTS AND DISCUSSION

3.1 Morphology characterization of the biosensors

The surface morphologies of (A) PANI, (B) GRA, (C) PANI-GRA and (D) PANI-GRA/PtNPs were characterized by scanning electron microscopic (SEM). The scanning electron microscopy images showed rod-like structure for PANI (Figure. 2A) and they tended to aggregate together to form networks which was suitable for biomolecule immobilization [34,35].

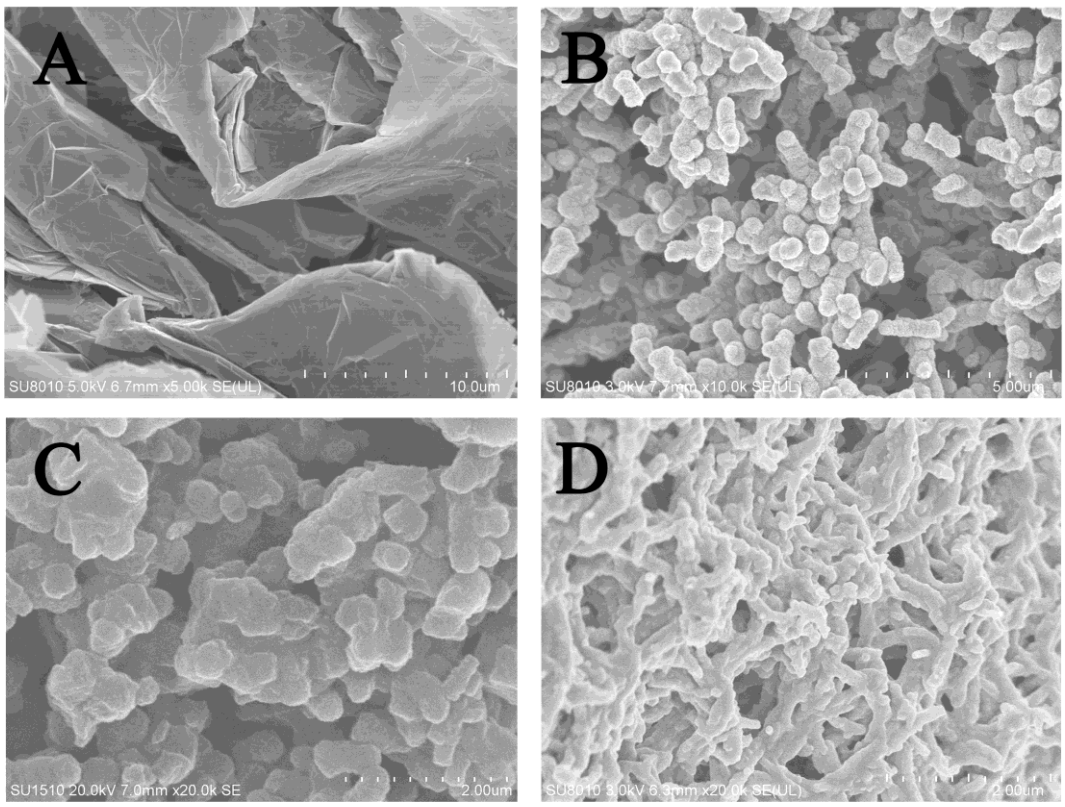

Figure 2. SEM images of (A) PANI, (B) GRA, (C) PANI-GRA, and (D) PANI-GRA/PtNPs.

GRA (Figure. 2B) exhibited a curved, flake-like and few-layers structure that was consistent with the results of Raman spectra [26]. This structure provided exceptional properties of excellent

physical isolation and superior conductivity. As indicated in Figure. 2C, there were significant changes in morphology when PANI and 2D GRA combined together. Instead of rod-like shapes, PANI showed a larger diameter but a shorter length. The morphology suggested that 2D GRA was enwrapped by PANI to form the nanocomposite [27]. The well-attached PANI-GRA nanocomposite could be ascribed to the π-π interaction between graphene surface and the conjugate structure of PANI [36,37]. Moreover, in situ electrochemical synthesis of aniline in the solution containing graphene which was fully dispersed with HCl, allowed PANI-GRA nanocomposite to homogeneously deposited on the electrode surface. PANI-GRA nanocomposite provided an excellent matrix for dispersing PtNPs, which expanded its usage in the construction of biosensor. It could be clearly seen from Figure. 2D that PtNPs were deposited on the surface of PANI-GRA nanocomposites and parts of them were further embedded in PANI-GRA nanocomposite, which might be affected by the conditions of the electrochemical synthesis [38]. The results suggested that an easy and controllable two-step electrochemical polymerization method employed in this work could be a satisfactory choice in fabricating three or more component nanomaterials.

3.2 Electrochemical characterization of the biosensors

In order to investigate the electrocatalytic effect of all these mentioned materials, various electrodes based on PANI/PtNPs, PANI-GRA/PtNPs, PANI-GRA/PtNPs/CS, PANI/PtNPs/CS-GOD and PANI-GRA/PtNPs/CS-GOD have been fabricated and investigated with cyclic voltammograms (CVs) electroanalytic methods in 0.02 M PBS (pH 6.5) at a scan rate of 50 mV/s. As shown in Figure. 3, no oxidation peak was observed between the absence and presence of 0.5 mM glucose at Pt/PANI/PtNPs (curve a and curve b), Pt/PANI-GRA/PtNPs (curve c and curve d) and Pt/PANI-GRA-PtNPs/CS (curve e and curve f) electrodes, which suggested that PANI/PtNPs, PANI-GRA/PtNPs and CS had no catalytic effects on the reaction of glucose. When GOD were embedded into the PANI/PtNPs and PANI-GRA/PtNPs nanocomposite film, the obvious oxidation peaks could be seen after adding glucose (curve b and curve d) compared with their respective CVs without glucose (curve a and curve c). The oxidation peaks might be assigned to catalytic oxidation of GOD occurred on the biosensor, which was attributed to the electron transfer process between the electroactive center and the electrode. Furthermore, comparing curve b with curve d, the peak current of the electrode coated with PANI-GRA/PtNPs was much higher than that of the electrode coated with PANI/PtNPs. In other words, the oxidation current obviously increased with the addition of 2D graphene. The results proved that 2D graphene with large surface area and fast charge transport ability could efficiently enhance the sensitivity of glucose biosensor. The reaction mechanism of glucose biosensor was described as follows [3].

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

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

The measurement of glucose was realized by amperometric monitoring of the production of hydrogen peroxide [39].

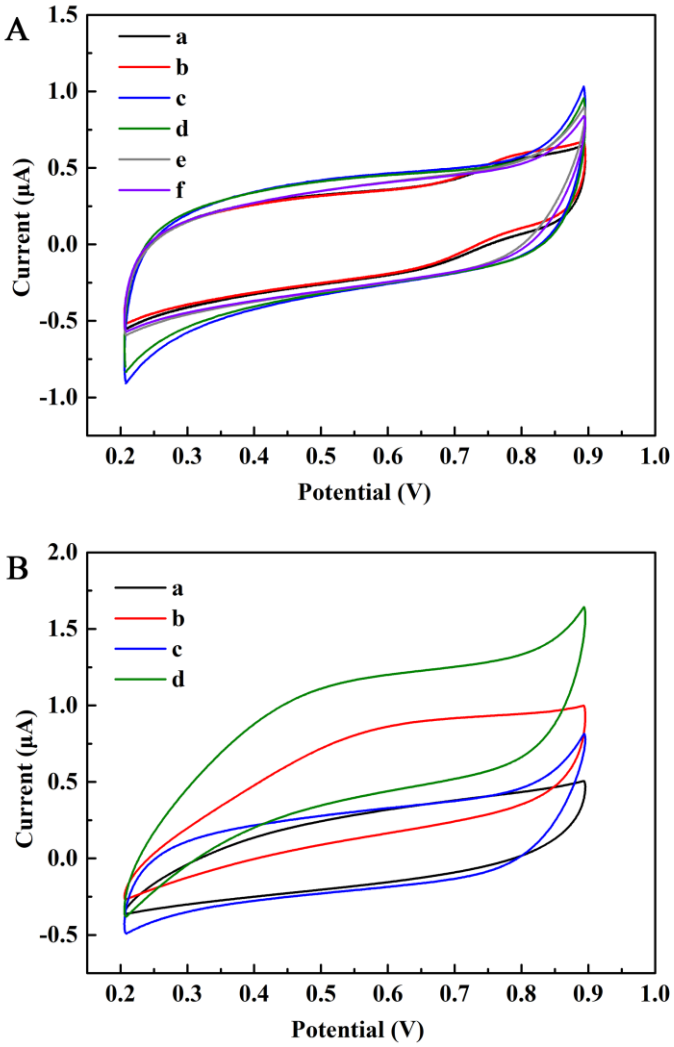

Figure 3. (A) CVs in 0.02 M phosphate buffer (pH 6.5) at 50 mV/s for (a) without glucose, and (b) with 0.5 mM glucose of Pt/PANI/PtNPs, (c) without glucose, and (d) with 0.5 mM glucose Pt/PANI-GRA/PtNPs, (e) without glucose, and (f) with 0.5 mM glucose Pt/PANI-GRA/PtNPs/CS. (B) CVs in 0.02 M phosphate buffer (pH 6.5) at 50 mV/s for (a) without glucose, and (b) with 0.5 mM glucose of Pt/PANI/ PtNPs/CS-GOD, (c) without glucose, and (d) with 0.5 mM glucose of Pt/PANI-GRA/PtNPs/CS-GOD.

3.3 Optimal conditions of the biosensor

The applied potential and pH value of solution are essential factors for the performance of the response current of PANI-GRA/PtNPs/CS-GOD biosensor. The effect of applied potential on response current to 0.5 mM glucose in 0.02 M phosphate buffer (pH 6.5) was presented in Figure. 4A. The response current increased as the applied potential stepped from 0.35 V to 0.55 V. However, when the applied potential moved from 0.55 V to 0.85 V, the response current gradually decreased. At 0.55 V, the biosensor reached a maximum current value. Therefore, an optimal applied potential of 0.55 V (vs. SCE) was preferred in the subsequent experiments.

Figure. 4B indicated the correlation of the response current and the pH values in 0.02 M phosphate buffer containing 0.5 mM glucose. The response current of the glucose biosensor increased with increasing pH value from 3.5 to 6.5, then reached a relatively stable level between 6.5 and 7.0, and finally decreased as the pH value further increased from 7.0 to 8.0. The response current of the biosensor was not sensitive to the change of pH compared with the change of applied potential, which may be attributed to the structure of 2D graphene that played a role as a barrier to buffer solution. There was a relative stable activity at pH 6.5, and therefore it was acceptable to select pH 6.5 as optimal pH value.

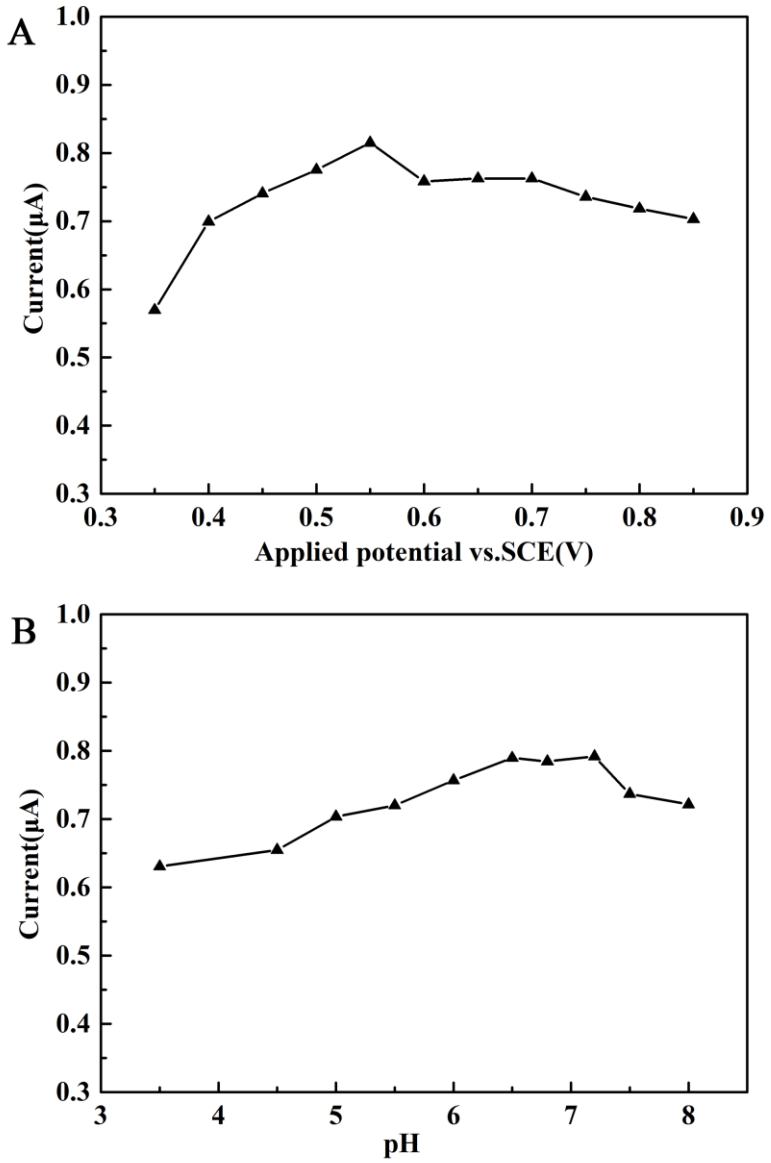

Figure 4. (A) Effects of applied potential on the response current of PANI-GRA/PtNPs/CS-GOD biosensor in 0.02 M phosphate buffer (pH 6.5) containing 0.5 mM glucose. (B) Effects of pH values on the response current of PANI-GRA/PtNPs/CS-GOD biosensor in different buffers containing 0.5 mM glucose.

3.4 Amperometric measurements of the biosensors

The amperometric responses of biosensors based on different nanocomposites were shown in Figure. 5A. The current signals of PANI-GRA/PtNPs/CS-GOD based biosensor (curve a) were about two times more highly than those of PANI/PtNPs/CS-GOD based biosensor (curve b), which could be contributed to superior conductivity of 2D GRA. Moreover, it was interesting to note that the sensitivity of PANI-GRA/PtNPs/CS-GOD biosensor significantly increased compared with PANI-GRA/CS-GOD biosensor reported in our previous literature [27], which was ascribed to the good electrocatalytic activity of PtNPs. The biosensor also exhibited a fast response time within 3s (98.3% of steady-state current).

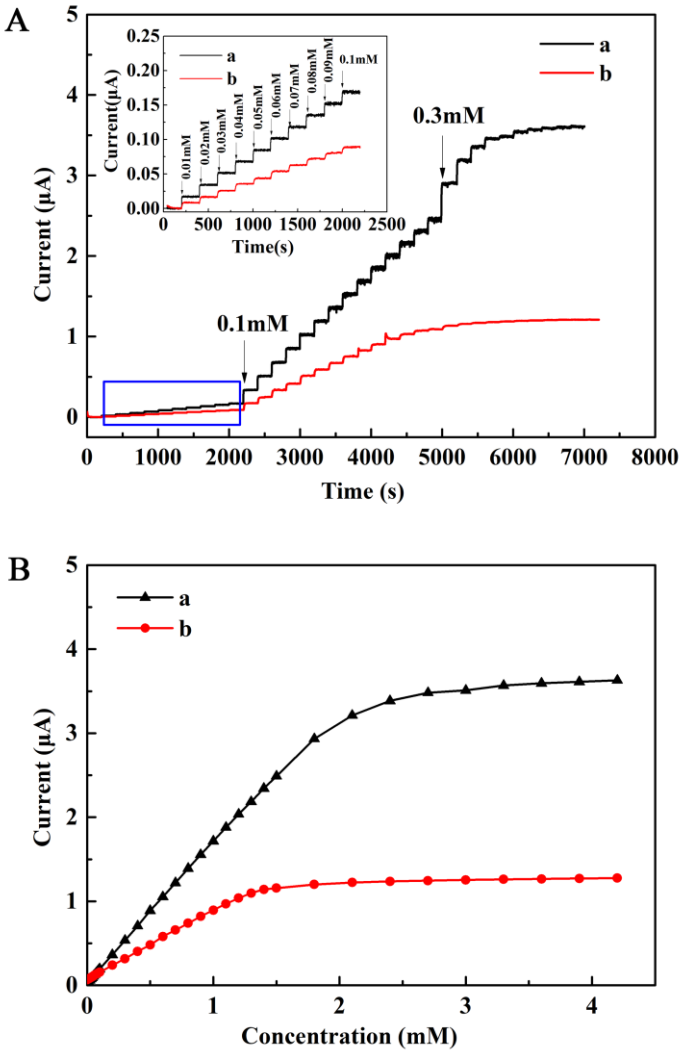

Figure 5. (A) Amperometric responses to successive injection of glucose at an applied potential of 0.55 V vs. SCE in 0.02 M phosphate buffer (pH 6.5) for (a) PANI-GRA-PtNPs/CS-GOD and (b) PANI/ PtNPs/CS-GOD biosensors. Inset: the magnified part of the curve marked with blue square. (B) Current-Concentration curves of (a) PANI-GRA/PtNPs/CS-GOD and (b) PANI/PtNPs/CS-GOD biosensors.

The results demonstrated the electrochemically synthesized PANI-GRA/PtNPs nanocomposite combined the electrical properties of 2D graphene and the strong catalytic activity of PtNPs. Thus, it accelerated electron transfer from enzyme to electrode, resulting in rapid and sensitive current signal toward the change of glucose concentration. Such electrodeposition method comprised of two fast and simple steps could be an ideal choice for the fabrication of nanocomposites in electroanalytical applications, since a large surface area and enhanced charge-transport properties of the nanocomposite material could be achieved.

Figure. 5B showed the relationship between response current and glucose concentration for both biosensors. In low-concentration region, the response current was linear with glucose concentration, indicating that the oxidation of glucose follows first-order reaction. While in relative high-concentration region, the response current increased slowly with further increasing glucose concentration, suggesting that the reaction followed zero-order reaction kinetic, which was the typical character of enzymatic reactions. The linear range spanned the concentration of glucose from 10.0 μM to 1.8 mM with a correlation coefficient of 0.9995. The sensitivity calculated from the linear portion of the calibration was 52.67 μA mM^{-1} cm^{-2}, which was much higher than those reported previously [27,29,40], but not as good as the results of other reported biosensors [19,20]. The biosensor offered a lower detection limit of 1.19 μM at a signal-to-noise ratio of 3. The apparent Michaelis-Menten constant was estimated to be 35.8 mM.

3.5 Selectivity and stability of the biosensor

A key factor of the sensor for accurate determination of target analyte is the selectivity. The common redox active interferents in human plasma, such as Glycine, D-galactose, ascorbic acid and L-tyrosine, especially L-phenylalanine and urea, are often electroactive in positive potential region and interfere with the detection of glucose. The experiments were performed with those interferences in the glucose amperometric determination to evaluate the selectivity of as-prepared biosensor. As shown in Figure. 6, the biosensor showed rapid and clear response to the addition of 0.2 mM glucose but no response occurred when 0.2 mM glycine, 0.2 mM Urea, 0.1 mM L-phenylalanine, 0.2 mM ascorbic acid, 0.2 mM D-galactose and 4.9 μM L-tyrosine were injected into the solution. The modified biosensor completely rejected six interfering species and presented outstanding anti-interference ability, which could be attributed to the excellent physical isolation of 2D graphene and the permselectivity of nanocomposite film. The selectivity of the biosensor in this work had advantages over previously reported glucose biosensors [19,20,27–29,40]. A further comparison of the performance of different electrochemical biosensors for the determination of glucose was summarized in Table1. With these excellent behaviors, the developed biosensor might be used for glucose detection in human plasma samples.

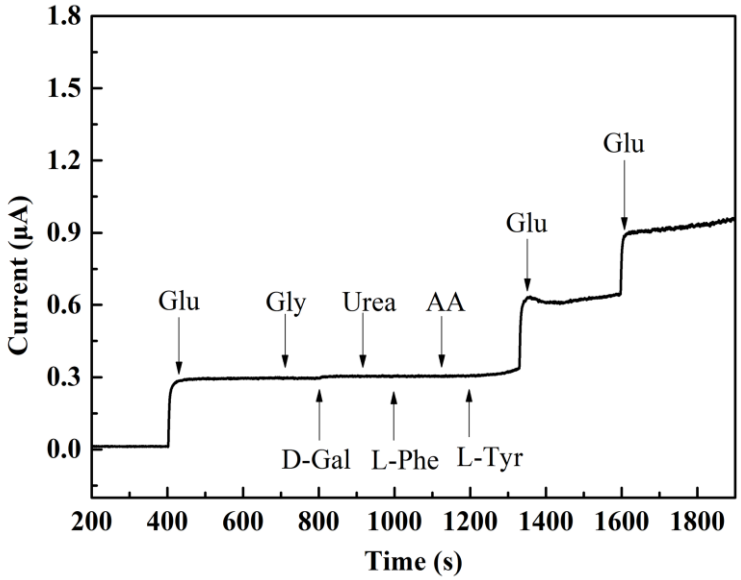

Figure 6. Effects of the interferences on the amperometric responses of the glucose biosensor in the presence of glucose.

Table 1. Comparison of characteristics of different glucose biosensors

Biosensor structure	Sensitivity (μA mM^{-1} cm^{-2})	Linear range (mM)	Response time (s)	Selectivity	References
PANI-GRA-AuNPs	-	0.004-1.12	8	High	[28]
PANI-GRA-AuNPs	20.32	0.2-11.2	-	Good	[29]
PANI/PtNPs	96.1	0.01-8	3	-	[19]
PANI-MWNT/PtNPs	128	0.003-8.2	5	High	[20]
PANI-BNNTs-PtNPs	19.02	0.01-5.5	3	-	[40]
PANI-GRA	22.21	0.01-1.48	5	High	[27]
PANI-GRA/PtNPs	52.67	0.01-1.80	3	Superior	This work

Abbreviations: MWNT: multi-wall carbon nanotube; BNNTs: wrapped boron nitride nanotubes.

The reproducibility of the biosensor was evaluated by comparing the response currents to 0.5 mM glucose at four fabricated biosensors under the same condition. The results revealed that the biosensor had satisfactory reproducibility with relative standard deviations (RSD) of 3.2%, owing to controllable electrochemical deposition method employed in the construction of biosensor. The operational stability of the biosensor was also investigated from the response currents in 0.2 mM and 0.4 mM glucose for 10 times, respectively. The RSD for 0.2 mM glucose was 2.35%, and for 0.4 mM glucose it was 1.92%.

The long-term stability of the biosensor was assessed by monitoring the response currents of the same electrode to 0.5 mM glucose every 7 days. When not in use, the electrode was stored in 0.02 M phosphate buffer solution (pH 6.5) at 4 ℃ in a refrigerator. The results showed the biosensor still retained 83% of its original values over four weeks, reflecting an acceptable long-term life of the

biosensor. The results implied that the PANI-GRA/PtNPs nanocomposite could effectively maintain the biocatalytic activity of GOD.

3.6. Determination of glucose in plasma samples

The practical application of the biosensor was assessed by the determination of glucose in human plasma samples. The samples were diluted about 200 times in 0.02M phosphate buffer (pH 6.5) and the response currents were measured. The glucose concentrations in the plasma samples were calculated according to the linear regression equation and were also listed in Table 2. The results were satisfactory and in good consistency with those obtained by BECKMAN-COULTER AU5800 biochemical analyzer in hospital. The recovery values ranged from 95.9% to 105.7%. The favorable results revealed that the glucose biosensor showed freedom of interferences and offered accurate determination of glucose in human plasma samples. Thus, the proposed biosensor could be applied to clinical detection of glucose.

Table 2. Determination of glucose in plasma samples

Sample Number	Determined by hospital (mM)	Determined by the sensor (mM)	Recovery (%)
1	3.64	3.51	96.4
2	3.88	3.72	95.9
3	5.60	5.73	102.3
4	5.61	5.83	103.9
5	11.28	11.76	104.3
6	12.68	13.40	105.7

4. CONCLUSIONS

In this work, the PANI-GRA/PtNPs nanocomposite have been successfully fabricated through in suit two-step electrochemical deposition method as a matrix for the adsorption of GOD. Compared with the chemical preparation method, the electrochemical polymerization process permitted the synthesis without any oxidizing agent, and the thickness of the film could be controlled. The developed glucose biosensor possessed superior sensitivity of 52.67 μA mM^{-1} cm^{-2} and selectivity, which showed the elimination of electroactive interferents from glycine (Gly), Urea (Urea), L-phenylalanine (L-Phe), ascorbic acid (AA), tyrosine (L-Tyr) and D-galactose (D-Gal), also fast response time due to 2D graphene and the synergistic effect with PtNPs, and it could be highly useful in the determination of glucose in real human plasma. In addition, electrochemical synthesis of nanocomposite material may have potential applications in the fabrication of other enzyme biosensors and provide a good platform for biosensing and biocatalysis.

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