

# Enhanced amperometric response of a glucose oxidase and horseradish peroxidase based bienzyme glucose biosensor modified with a film of polymerized toluidine blue containing reduced graphene oxide

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**Abstract** Reduced graphene oxide (RGO) was used to construct a bienzyme biosensor containing horseradish peroxidase (HRP) and glucose oxidase (GOx). A poly(toluidine blue) (pTB) film containing RGO acted as both enzyme immobilization matrix and electron transfer mediator. The bienzyme biosensor was characterized by electrochemical techniques and displays a highly sensitive amperometric response to glucose and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at a potential as low as -0.1 V (vs. SCE). It is shown that use of RGO causes a strong enhancement on the amperometric responses. H<sub>2</sub>O<sub>2</sub> formed by the action of GOx in the presence of oxygen can be further reduced by HRP in the pTB film contacting the RGO modified electrode. In the absence of oxygen, glucose oxidation proceeds by another mechanism in which electron transfer occurs from GOx to the electrode and with pTB acting as the mediator. Amperometric responses to glucose and H<sub>2</sub>O<sub>2</sub> follow Michaelis-Menten kinetics. The experimental conditions were optimized, and under these conditions glucose can be determined in the 80 μM to 3.0 mM range with a detection limit of 50 μM. H<sub>2</sub>O<sub>2</sub>, in turn, can be quantified in up to 30.0 μM concentration with a detection limit of 0.2 μM. The bienzyme biosensor is reproducible, repeatable and stable. Finally, it has been successfully applied to the determination of glucose in plasma samples.

**Keywords** Reduced graphene oxide · Bienzyme biosensor · Horseradish peroxidase · Glucose oxidase · Toluidine blue · Amperometry;

## Introduction

There has been an explosion of interest in the use of nanomaterials for the development of biosensors, and carbon nanotubes (CNTs) are at the forefront [1]. As the thinnest but also the hardest nanomaterial, now graphene has attracted more and more attention since it was first reported in 2004 [2, 3]. However, the dispersibility of graphene in solvents is not good due to its hydrophobic nature, which is limited to its application in electrochemical analysis. To improve its dispersibility, some functional graphene have been synthesized and widely used in electrochemical analysis [4–6]. Most of them used in electrochemistry are produced by chemically reduction from graphene oxide (GO). Both GO and reduced graphene oxide (RGO) usually have abundant structural defects [7, 8] and functional groups [9] which are advantageous for electrochemical applications [4, 10, 11]. In 2009, Dong revealed that RGO as a kind of more robust and advanced carbon electrode material holds great promise for electrochemical biosensor design because RGO consists of a single sheet showing favorable electrochemical activity [5]. Up to now, many RGO-based electrochemical sensors and biosensors have been constructed for biochemical analysis such as the determination of a neurotransmitter dopamine (DA) and its coexisting species ascorbic acid (AA) and uric acid (UA) [12, 13], a cofactor of many dehydrogenases NADPH and a general enzymatic product of oxidases and a substrate of peroxidase H<sub>2</sub>O<sub>2</sub> [14, 15]. Besides that, heavy metal ions and even DNA have been successfully analyzed by RGO-based biosensors [16, 17].

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Enzyme biosensor continues to be a topic of interest in the field of electroanalytical techniques and devices because enzyme has high selectivity and respond quickly to the specific substrate [18]. Much attention has been paid to the development of high-performance enzyme sensors. As we know, enzyme activity is easily influenced in amperometric biosensors by operation conditions such as immobilization methods, electrode materials, electrolyte pH and temperature. So the establishment of electrical communication between redox centers of enzymes and common electrode is thought as key factor determining the performance of biosensor because the redox active site of enzyme is usually embedded deeply into the large three dimensional structure of enzyme molecules. Based on this consideration, many nanomaterials were applied to the enzyme-based biosensors to increase the conductivity and compatibility in terms of their unique chemical, electronic and mechanical properties [19–21]. Unsurprisingly, RGO with high electrocatalytic activity has also been involved in the preparation of enzyme biosensors due to its rich oxygen-containing functional groups and good dispersibility in water. Kinds of RGO-based glucose biosensors have been reported [22–27]. The high surface area is helpful in increasing the surface loading of the target enzyme molecules on the surface. The excellent conductivity and small bandgap are favorable for conducting electrons from the biomolecules [19]. In addition to using nanomaterials for electrical communication between redox centers of enzymes and electrode, the entrapment of enzymes in electropolymerized conductive films shows high efficiency in fabrication of biosensors due to simplicity of operation and low response time. Generally, electropolymerized films exhibit good uniformity and controllability. Moreover, some of them can also work as electron mediator to accelerate the electron transfer rate between enzyme and electrode. The aim of our work is the development of a bienzyme biosensor combining unique electronic properties of RGO and good uniformity and controllability of electropolymerized films for glucose or  $\text{H}_2\text{O}_2$  determination.

In present work, one bienzyme-based amperometric biosensor with both glucose oxidase (GOx) and horseradish peroxidase (HRP) enzymes co-immobilized in ploy(toluidine blue) (pTB) film has been developed and characterized by electrochemical method on RGO modified GC electrode and its amperometric response for glucose and  $\text{H}_2\text{O}_2$  at low potential was well-behaved with displaying Michaelis-Menten kinetics. RGO showed great enhancement effect on amperometric responses of this biosensor. As a two-electron redox mediator, toluidine blue (TB) also act as the polymer matrix to immobilize the enzymes remaining biologic activity under this condition. This biosensor offered high sensitivity, good selectivity and fast response. Finally, the biosensor was successfully applied to the glucose determination in plasma sample.

## Material and methods

### Chemicals and apparatus

Glucose oxidase (EC 1.1.3.4, type X-S, from *Aspergillus niger*, GOx), peroxidase (EC 1.11.1.7, type VI, from horseradish, HRP), toluidine blue (TB), ascorbic acid (AA), uric acid (UA), L-aspartic acid (L-Asp), L-tryptophan (L-Trp),  $\gamma$ -aminobutyric acid (GABA), L-Lysine (L-Lys) and polyvinylpyrrolidone (PVPy,  $M_w=40,000$ ) were obtained from Aladdin reagent Co. (Shanghai, China, [www.aladdin-e.com](http://www.aladdin-e.com)), and used as received. Graphite oxide was synthesized from graphite (spectral pure, Sinopharm Chemical Reagent CO., China, [en.reagent.com.cn](http://en.reagent.com.cn)) by Hummers method [28]. The glucose and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were purchased from Sinopharm Chemical Reagent CO. (Shanghai, China). The stock solution of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was freshly prepared daily by diluting 30 %  $\text{H}_2\text{O}_2$  solution. Glucose stock solution was allowed to mutarotate for at least 24 h before use. Phosphate buffer (0.10 M,  $\text{pH}=7.4$ ) was prepared by mixing stock standard solutions of  $\text{KH}_2\text{PO}_4$ , KCl and NaOH. Other chemicals were of reagent-grade and were used as supplied without further purification. All solutions were prepared with deionized water.

All the electrochemical measurements were performed on a CHI 660D electrochemical workstation (CH Instruments, Chenhua, Shanghai, China, [www.chinstr.com](http://www.chinstr.com)) in a conventional three-electrode arrangement, equipped with a platinum wire counter electrode, a saturated calomel electrode (SCE) reference electrode and the modified glassy carbon working electrode (GCE,  $\phi=3.0$  mm). For the deoxygenated experiments, the electrolyte was bubbled with highly-purity (99.999 %) nitrogen for 15 min and the electrochemical experiments were conducted under inert atmosphere. All electrochemical experiments were carried out at room temperature. The scanning electron microscopy (SEM) was performed on LEO-1530 scanning electron microscope (LEO Electron Microscopy Inc., Cambridge, UK, [www.smt.zeiss.com/nts](http://www.smt.zeiss.com/nts)).

### Synthesis of reduced graphene oxide

Reduced graphene oxide (RGO), was prepared according to references [22, 29]. Synthesized GO was suspended in water to create a  $0.50 \text{ mg}\cdot\text{mL}^{-1}$  dispersion. Exfoliation of GO was achieved by ultrasonication of the dispersion for 30 min. Then obtained brown dispersion was subjected to 30 min of centrifugation at 330 g to remove any unexfoliated GO. The homogeneous GO dispersion (5.0 mL) was mixed with 5.0 mL of  $4 \text{ mg}\cdot\text{mL}^{-1}$  polyvinylpyrrolidone (PVPy) aqueous solution, and then stirred at  $50^\circ\text{C}$  for 12 h. When it was cooled to room temperature,  $3.5 \mu\text{L}$  of hydrazine and  $40.0 \mu\text{L}$  of ammonia solutions were added into the dispersion. After being vigorously shaken or stirred for a few

minutes, the vial was put in an oil bath (95 °C) for 1 h. The homogeneous black dispersion was obtained and then washed to neutrality by repetitive centrifugation and resuspension. The final volume of aqueous RGO dispersion was 2.5 mL.

### Fabrication of the biosensor

Prior to preparation of modified electrode, a glassy carbon electrode (GCE) of 3.0 mm was mechanically polished to a mirror finish with wet microcloth containing 0.05  $\mu\text{m}$  alumina powder, and then carefully cleaned in ethanol ( $\text{C}_2\text{H}_5\text{OH}$ ) and water by ultrasonication bath, each for 2 min. 8.0  $\mu\text{L}$  of the uniform RGO suspension was cast on the GCE surface and air dried for several hours. Electropolymerization of TB on the RGO modified GCE (GCE/RGO) was carried out by cyclic voltammetry between  $-0.40$  and  $+0.90$  V at a scan rate of  $50 \text{ mV}\cdot\text{s}^{-1}$  for 20 cycles in phosphate buffer ( $\text{pH}=7.4$ ) [23]. The concentration of TB is 5.0 mM. The horseradish peroxidase (HRP) - glucose oxidase (GOx) bienzyme electrode was prepared via electropolymerization of TB in the presence of HRP and GOx. It was signed as GCE/RGO/pTB-HRP-GOx. The modified electrodes were completely rinsed by deionized water and then stored in pH 7.4 phosphate buffer at 4 °C, when not in use.

### Amperometric measurement of glucose or $\text{H}_2\text{O}_2$

Amperometric measurements of glucose or  $\text{H}_2\text{O}_2$  were performed in a 0.10 M phosphate buffer ( $\text{pH}=7.4$ ) at the applied potentials of  $-0.10$  V. The steady-state current response was recorded after the addition of glucose or  $\text{H}_2\text{O}_2$  in stirred solution.

## Results and discussion

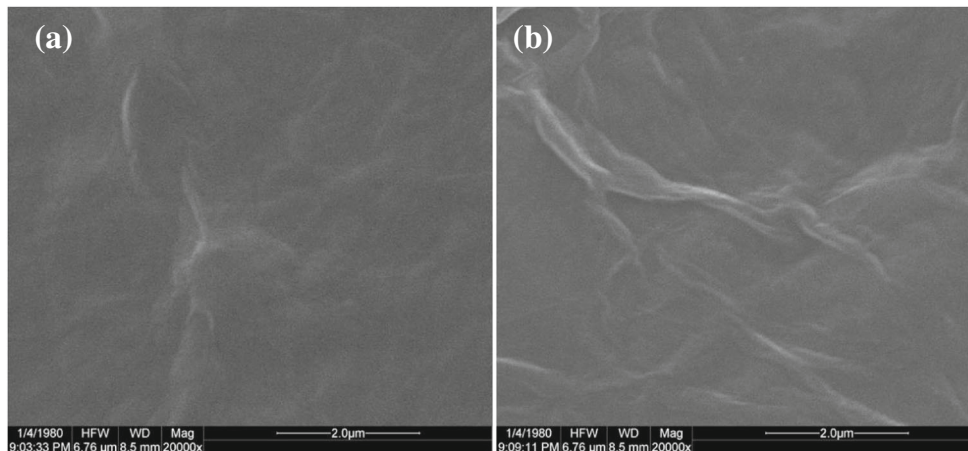
### SEM images

The RGO modified glassy carbon electrode (GCE/RGO) and the bienzyme biosensor based on this chemically modified electrode (GCE/RGO/pTB-HRP-GOx) were characterized using scanning electron microscopy (SEM). The SEM images of both GCE/RGO and GCE/RGO/pTB-HRP-GOx are respectively shown in Fig. 1a and b. It can be found that the RGO shown like layer film with some wrinkles has no aggregations, indicating that RGO can be uniformly dispersed in water (Fig. 1a). The GCE/RGO has high surface area offering much more active sites than the bare GC electrode. Combined with the special electronic characteristics of RGO, this film can be regarded as good carrier of bienzyme HRP and GOx. As shown in Fig. 1b, a very thin film appears and overlies on the RGO surface, suggesting that HRP and GOx have been successfully entrapped in the conductive pTB film on RGO sheets which have tightly attached to the electrode surface. RGO film with wrinkles can offer high accessible surface area to increase the loading of enzyme and then enhance the amperometric responses of corresponding substrates.

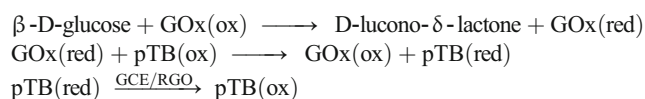
### Biocatalytic schemes

Bienzyme electrode was fabricated by co-immobilizing GOx and HRP onto the RGO modified GCE via electropolymerization of toluidine blue (TB) as shown in Scheme 1c. The redox center of enzyme far away from the electrode surface can “contact” the modified electrode with the aid of conductive film pTB and RGO, resulting in fast responses of biosensor to corresponding substrates. There are two routes involved in this bienzyme biosensor for glucose detection. In the first route (Scheme 1b), the conductive pTB film acts as mediator to achieve the electrical connection between GOx and GCE/RGO without participation of  $\text{O}_2$  [23, 30]. Amperometric

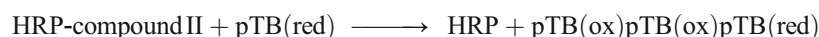
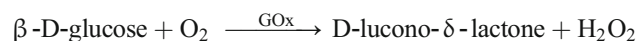
**Fig. 1** SEM views with the same magnification: (A) RGO film; (B) RGO/pTB-HRP-GOx film



response for glucose detection displayed as anodic current via monitoring the oxidation of glucose by GOx.



As for the second route (Scheme 1a), the principle of present bioenzyme electrode is based on electrochemical reduction of the hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) generated by



Considering practicability and convenience of the biosensor, only the second route was used for further investigation on its electrochemical performance. Following this principle, the cathodic current response can be observed when some amount of glucose was injected in supporting electrolyte.

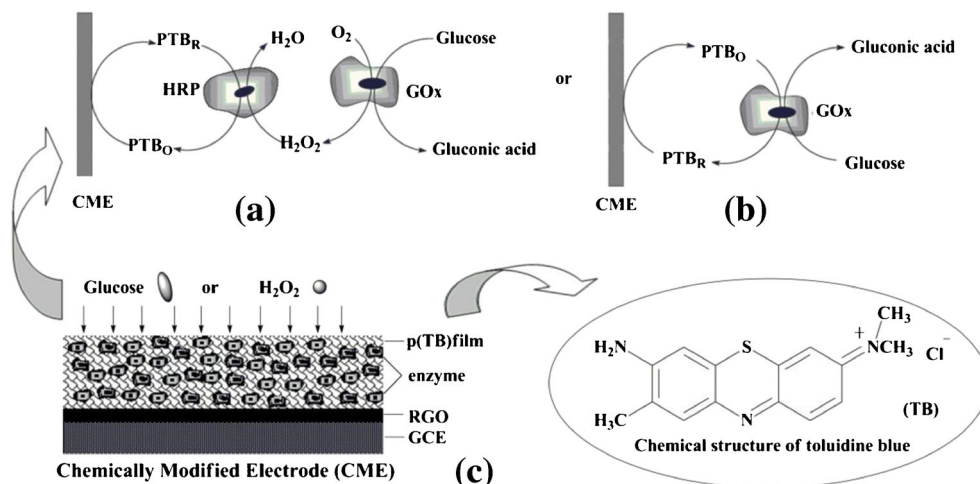
### pTB coating on bare GCE and GCE/RGO

The electropolymerization process was carried out by cyclic potential sweep from  $-0.40$  to  $+0.90$  V at a scan rate of  $50 \text{ mV}\cdot\text{s}^{-1}$  in phosphate buffer (pH 7.4) containing  $5.0 \text{ mM}$  TB in the presence of HRP and GOx. TB species were used as not only enzyme-immobilization material but also electron transfer mediator and showed different cyclic voltammetric behaviors at between bare GCE and GCE/RGO surface. The results are illustrated in Fig. 2.

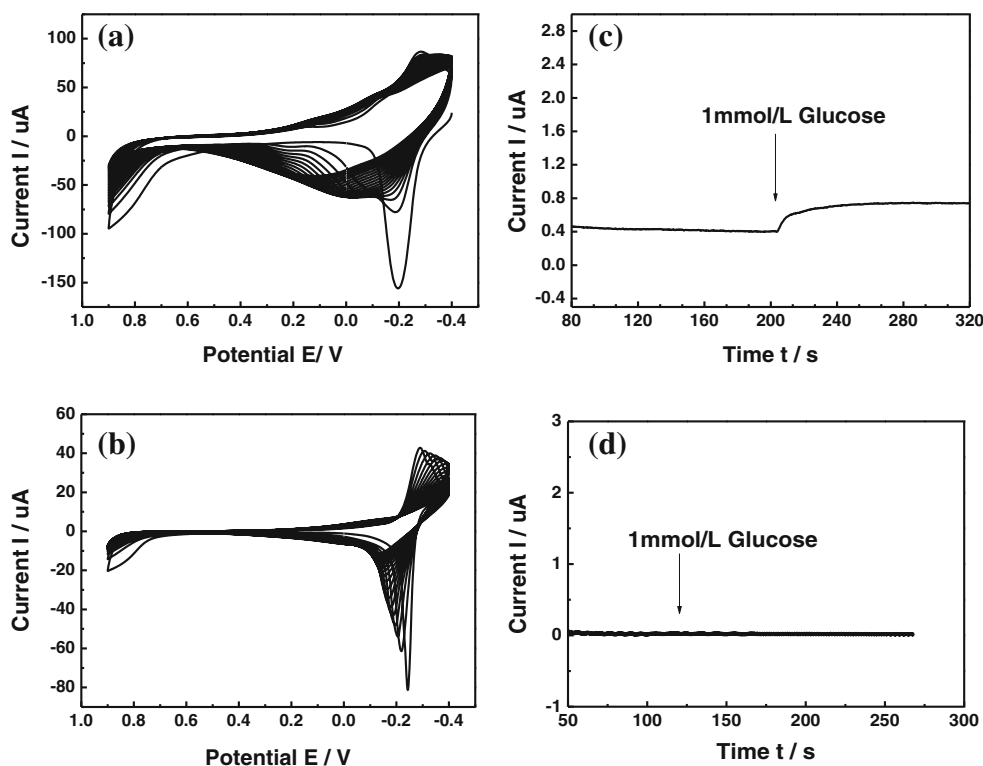
the enzymatic reaction in the presence of  $\text{O}_2$ . Active oxidants compound I was formed in catalytic reaction of HRP with  $\text{H}_2\text{O}_2$  and then it was reduced to be compound II by pTB film. Compound II was further reduced to generate the HRP by accepting a second electron from pTB film [30, 31]. In this bienzymatic system, pTB film serves as electrons donor to contribute to the generation of ferric enzyme HRP. The corresponding details can be described as follows:

The electropolymerization of TB at graphite electrode has been reported in details [30] and but shows a slightly different here. At GCE/RGO (Fig. 2a), a well-defined oxidation peak with large current can be observed at about  $-0.20$  V when potential initially sweeps from  $-0.40$  to  $+0.90$  V. Corresponding reduction peak can be observed at about  $-0.30$  V on reverse scan. Maybe it was relevant to the adsorption of TB species onto electrode surface and then the redox of TB monomers. On the following potential scans the polymer of TB is formed in RGO film and the redox peak currents of TB monomer decrease slowly and disappear at the last. At the same time, a new couple of redox peaks appear at about  $0.00$  and  $-0.10$  V with the growth of polymer film, which is attributed to oxidation and reduction of generated intermediate compounds in process of electropolymerization of TB [30]. The less obvious shoulder redox peaks at about  $0.20$  V and  $0.10$  V appear based on the same reason. Similar redox process of TB

**Scheme 1** Schematic representation of glucose detection at GCE/RGO/pTB-HRP-GOx



**Fig. 2** Cyclic voltammograms for electropolymerization of TB in the presence of HRP and GOx at GCE/RGO (a) and bare GCE (b), and corresponding amperometric responses to 1.0 mM glucose of GCE/RGO/pTB-HRP-GOx (c) and GCE/pTB-HRP-GOx (d). Scan rate:  $50 \text{ mV} \cdot \text{s}^{-1}$



monomers can be obtained at bare GCE and but the other two couple of redox peaks from intermediate compounds are almost not found during the following voltammetric sweeps (Fig. 2b). So it can be concluded that the electronic properties of polymerized TB film at RGO are more appropriate than that at bare GCE for biosensor construction. It was the large surface area and special electronic characteristics of RGO film that greatly enhance the polymerization rate of TB and the amount of enzyme (HRP and GOx) entrapped in pTB film. Amperometric response currents in Fig. 2c and d have confirmed the above conclusion. At the same glucose concentration of 1 mM, the current response at GCE/RGO (Fig. 2c) is much larger than that obtained at bare GCE (Fig. 2d). These results indicated that the cooperation of RGO film and pTB film meant a great deal to the high-performance of this bienzyme biosensor.

### PTB film thickness

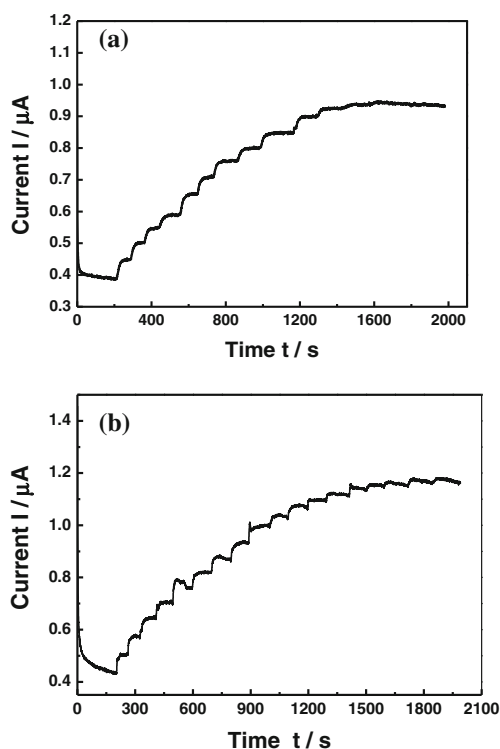
As an immobilization matrix for GOx and HRP, thin pTB film is beneficial to the diffusion of substrates or products and but amount of enzyme loading will decrease. Meanwhile, if pTB film is a redox mediator for electron transfer between active sites of enzyme and electrode, thick film will benefit the performance of this biosensor. Therefore, effect of pTB film thickness controlled by the number of voltammetric cycles

on current response of glucose was investigated in the range of 5 to 25. The current response increased greatly from 5 to 15 cycles and then remained stable after 20 cyclic potential sweeps. So electropolymerization of TB was carried out at 20 cyclic potential sweeps.

### Amperometric response of the bienzyme biosensor to glucose and $\text{H}_2\text{O}_2$

Amperometric currents of GCE/RGO/pTB-HRP-GOx to glucose and  $\text{H}_2\text{O}_2$  are respectively recorded in Fig. 3a and b at a detection potential of  $-0.10 \text{ V}$  in air-saturated phosphate buffer.

It can be found that the currents increase with successive addition of glucose and  $\text{H}_2\text{O}_2$  and then finally they reach a saturation value. The response was very fast and the current reached steady state within 5 to 8 s. As for the glucose determination, the response current would reach a saturated value when the concentration of glucose was higher than 4.0 mM. If the concentration was further increased, the response signals would change to be anodic current, which may be influenced by the concentration of dissolved  $\text{O}_2$  in buffer solution. After the substrate was added in solution, the  $\text{O}_2$  near the electrode surface was consumed to produce  $\text{H}_2\text{O}_2$  during enzymatic oxidation of glucose. If the  $\text{O}_2$  in bulk solution did not diffuse to the electrode surface in time, the enzymatic oxidation of



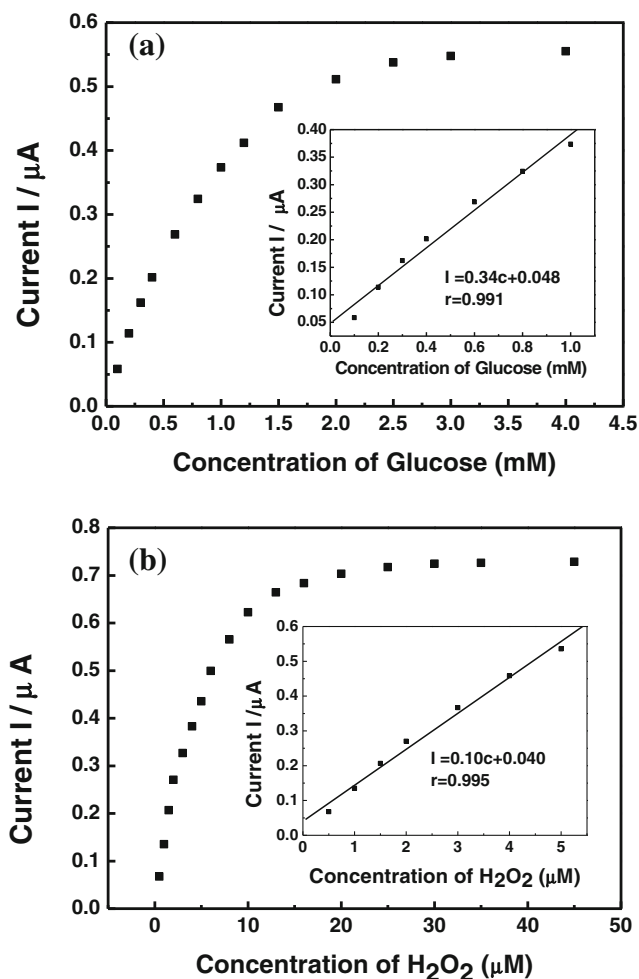
**Fig. 3** Amperometric response of GCE/RGO/pTB-HRP-GOx to successive addition of glucose (a) and H<sub>2</sub>O<sub>2</sub> (b) in air-saturated phosphate buffer (pH 7.4) at a detection potential of  $-0.10$  V under steady-state conditions

glucose would proceed by the other route in (Scheme 1b) utilizing the mediator role of pTB film on GCE/RGO and production of H<sub>2</sub>O<sub>2</sub> would become slow, and lastly the current response changed. Anyway, the linear range of this enzyme electrode extended to 1.0 mM of glucose (Fig. 4a). The detection limit of this biosensor was estimated to 0.05 mM at a signal-to-noise of 3 ( $S/N=3$ ). Similarly, the H<sub>2</sub>O<sub>2</sub> response is shown in Fig. 4b. The linear range is up to 5.0  $\mu$ M and the detection limit was estimated to be 0.2  $\mu$ M ( $S/N=3$ ). This bienzyme electrode showed a typical Michaelis-Menten kinetics [32], as it was shown in Fig. 4. According to the Lineweaver-Burk equation:

$$1/I_{ss} = 1/I_{max} + K_m^{app}/c \cdot I_{max}$$

where,  $I_{ss}$  is the steady-state current after the addition of glucose,  $c$  is the bulk concentration of the substrate, and  $I_{max}$  is the maximum current measured under saturated glucose conditions, the apparent Michaelis-Menten constant  $K_m^{app}$ , can be derived to be 1.79 mM according to the Lineweaver-Burke plot ( $1/c$  vs.  $1/I$ ).

As for the responses of bienzyme biosensor to H<sub>2</sub>O<sub>2</sub>, the data were illustrated in Fig. 4b. In the same way, the  $K_m^{app}$  for the catalytic reaction of HRP to H<sub>2</sub>O<sub>2</sub> was calculated to be 6.34  $\mu$ M. The low  $K_m$  values indicated high

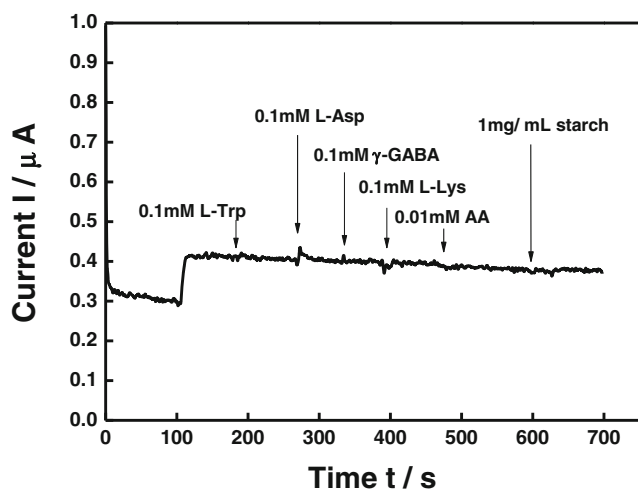


**Fig. 4** Plots of current responses vs. the concentration of glucose (a) and H<sub>2</sub>O<sub>2</sub> (b). The insets are corresponding calibration curve for glucose (a) and H<sub>2</sub>O<sub>2</sub> (b)

enzymatic activity of HRP and GOx in pTB film. This bienzyme system exhibited high biological affinity to glucose and H<sub>2</sub>O<sub>2</sub>, and electropolymerized TB film on RGO modified electrode provided appropriate environment for Michaelis-Menten equilibrium.

### Biosensor performance

A low detection potential of  $-0.10$  V was chosen for glucose determination, which can greatly reduce the interference from some small biological molecules that usually present in the typically samples. As it shown in Fig. 5, common interferences by some small biological molecules such as ascorbic acid (AA) and uric acid (UA, 0.01 M, not shown) have been greatly weakened on the response of this biosensor. Some kinds of amino acids including L-tryptophan (L-Trp), L-aspartic acid (L-Asp),



**Fig. 5** Amperometric response of GCE/RGO/pTB-HRP-GOx to 1.0 mM glucose in the presence of some co-existing compounds in phosphate buffer (pH=7.4), applied potential: -0.10 V

L-lysine (L-Lys) and  $\gamma$ -aminobutyric acid (GABA) have almost no influence on the performance of biosensor.

Reproducibility and repeatability are important factors for evaluation of biosensor performance. In present work, reproducibility and repeatability of fabricated sensor were investigated by manufacture of two biosensors at a time and 5 times repeat of detection. The relative standard deviation (RSD) for 5 times parallel detection of each sample including glucose or H<sub>2</sub>O<sub>2</sub> was less than 5.0 %. The RSD for 5 times parallel detection of 0.1 mM glucose and 0.01 mM H<sub>2</sub>O<sub>2</sub> is calculated as 4.8 % and 3.4 %, respectively. These data suggest excellent reproducibility and repeatability of this bienzyme biosensor.

In addition, the stability of this bienzyme biosensor was tested in 0.10 M phosphate buffer (pH=7.4) containing 1.0 mM glucose. The current response was deteriorated gradually and maintained at a level of about 70 % of the original after a week and about 50 % after 2 weeks, which may be resulted by the denaturation of HRP and

GOx. Also, the performance of this biosensor is compared with other RGO based glucose biosensors. The results are illustrated in Table 1. The fabricated biosensor for glucose determination shows a wider linear range. The beauty of this sensor is the low applied potential of -0.10 V which can not only reduce the interferences from coexisting biomolecules in sample at high potential but also can avoid the effect of oxygen reduction at cathodic potential. Lastly, our biosensor has been successfully applied to the real sample analysis. The glucose concentration of 5.7 mM in plasma sample confirmed reliability of this method.

### Conclusions

An amperometric biosensor for glucose and H<sub>2</sub>O<sub>2</sub> determination was developed by co-immobilization HRP and GOx immobilized in pTB film on RGO modified glassy carbon electrode. Thanks to RGO film with special electronic characteristics and pTB film as electron transfer mediator, this biosensor performed sensitive detection of glucose and H<sub>2</sub>O<sub>2</sub> at a very low potential of -1.0 V (vs. SCE), which can greatly reduce the interference from ascorbic acid (AA), uric acid (UA, 0.01 M) L-tryptophan (L-Trp), L-aspartic acid (L-Asp), L-lysine (L-Lys) and  $\gamma$ -aminobutyric acid (GABA). Moreover, amperometric responses of glucose and H<sub>2</sub>O<sub>2</sub> were well-behaved with displaying Michaelis-Menten kinetics. The Low K<sub>m</sub> values indicated that high enzymatic activity of HRP and GOx can be maintained in pTB film, suggesting that this bienzyme system exhibited high biological affinity to glucose and H<sub>2</sub>O<sub>2</sub>. Under optimized experimental conditions glucose can be determined in the 80  $\mu$ M to 3.0 mM range with a detection limit of 50  $\mu$ M. H<sub>2</sub>O<sub>2</sub>, in turn, can be quantified in up to 30.0  $\mu$ M concentration with a detection limit of 0.2  $\mu$ M. Based on wide linear range, good stability and reproducibility of the biosensor, it was applied to glucose determination in real sample. The result of 5.7 mM in plasma sample was satisfactory and confirmed reliability of this method.

**Table 1** The comparison of our biosensor with others in terms of performance

Material/method used	Linear range (mM)	Detection limit ( $\mu$ M)	K <sub>M</sub> <sup>app</sup> (mM)	Ref.
GC/RGO, PTB/GOx-HRP film, in 0.10 M phosphate buffer (pH 7.4) at - 0.10 V vs. SCE	0.08–1	50	1.79	this work
GC/RGO, Pd-GOx film, in 0.1 M phosphate buffer (pH 5.0) at - 0.45 V vs. SCE	up to 0.1	34	0.06	[24]
GC/RGO, Ag-GOx film, in 0.1 M phosphate buffer at pH 7.0 at -0.50 V vs. Ag/AgCl	0.5–12.5	160		[25]
GC/RGO-IL, AuNP-GOx film, in 0.1 M phosphate buffer (pH 7.4) at 0.35 V vs. SCE	2–18	130		[26]
GC/RGO, PB/PTBO/CHIT-GOD film, in 0.05 M phosphate buffer at pH 7.4, at 0.20 V vs. Ag/AgCl	0.02–1.09	8.4		[27]
GC/RGO, PAMAM–Ag film, in 0.1 M PBS (pH 7.0) at -0.25 V vs. Ag/AgCl.	0.032–1.89	4.5		[33]

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