



A glucose biosensor based on TiO₂–Graphene composite

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ABSTRACT

A novel glucose biosensor was developed based on the adsorption of glucose oxidase at a TiO₂–Graphene (GR) nanocomposite electrode. A TiO₂–GR composite was synthesized from a colloidal mixture of TiO₂ nanoparticles and graphene oxide (GO) nanosheets by an aerosol assisted self-assembly (AASA). The particle morphology of all TiO₂–GR composites was spherical in shape. It was observed that micron-sized TiO₂ particles were encapsulated by GR nanosheets and that the degree of encapsulation was proportional to the ratio of GO/TiO₂. The amperometric response of the glucose biosensor fabricated by the TiO₂–GR composite was linear against a concentration of glucose ranging from 0 to 8 mM at −0.6 V. The highest sensitivity was noted at about 6.2 μA/mM cm². The as prepared glucose biosensor based on the TiO₂–GR composite showed higher catalytic performance for glucose redox than a pure TiO₂ and GR biosensor.

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1. Introduction

Glucose biosensors are a common item of concern among all the enzyme-based biosensors due to their importance in the monitoring of blood glucose for the treatment and control of diabetes (Wang, 2008). Glucose oxidase (GOD) is employed as the enzyme in most glucose biosensors. The general principle of the glucose biosensor is based on the amperometric detection of H⁺ via the following chemical reactions (Kuila et al., 2011):



Glucose is the substrate of GOD, which will result in an enzyme-catalyzed reaction and decrease the concentration of GOD-FAD on the electrode surface. GOD assembled materials exhibit a fast electron transfer reaction on the electrode. Its oxidized form, FAD, is electrochemically oxidized at the electrode surface producing its reduced form, FADH₂ (Wu et al., 2010).

Graphene (GR) exhibits a unique structure of a two-dimensional (2D) nanosheet composed of hexagonal arrayed sp² hybridized carbon atoms with one-atomic thickness (Liu et al., 2011). The unique structure of GR instills in its fast electron

transportation, a high specific surface area, high thermal conductivity, excellent mechanical stiffness and good biocompatibility, which result in promising applications in nanocomposites, electromechanical resonators, solar cells and electrochemical sensors (Li et al., 2008).

Chen et al. (2010) developed Nafion-GR/GOD film-modified electrodes for the detection of glucose, which showed good stability. Shan et al. (2010) reported a good amperometric response to glucose with wide linear ranges using chitosan-GR/Au nanocomposite films for glucose sensing applications. Liu et al. (2011) synthesized self-assembled graphene platelet-glucose oxidase nanostructures for glucose biosensing applications. Thus, GR is expected to play an important role in improving the catalytic performance of biosensing applications due to its unique properties.

Many types of materials, such as SiO₂, gold, silver, CeO₂, MnO₂ and TiO₂ nanoparticles, have been used to construct nano-biosensors (Rahman et al., 2010). Among them, TiO₂ nanoparticles have attracted considerable interest due to the superior properties, such as their large specific surface area, high uniformity, and excellent biocompatibility (Li et al., 2009). Bao et al. (2008) reported that modified TiO₂ on an electrode could enhance the application of a glucose biosensor by the immobilization of the enzyme. They hydrothermally synthesized a new slack TiO₂ layer with a uniform porous nanostructure through the use of a MWNT template. They showed that TiO₂ provided the enzyme with a better immobilization environment because it enlarged the specific surface area. However, few studies have investigated TiO₂

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nanoparticles with GR nanosheets as an electrode material for glucose biosensors.

In the present study, we present an enhanced glucose biosensor based on the TiO_2 -GR composite. The TiO_2 -GR composite was synthesized by evaporating droplets composed of TiO_2 nanoparticles, GO and water by an aerosol-assisted self-assembly (AASA) method. The AASA method is of interest as it has many advantages; it is a simple system, it is cost-effective, and it can be scaled up to very high quantities (Jang et al., 2008).

GO can be reduced to form chemically modified GR in which the oxygen functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{O}-$) are removed by reduction reactions with chemical reducing agents such as hydrazine or by heat treatment in a reducing atmosphere (Cote et al., 2009a). However, the use of chemical reducing agents requires much time to prepare the composites due to many types of chemical reaction steps, long reaction time, necessity of repeated washing, and long drying time. Moreover, the process requires the use of poisonous and potentially explosive chemicals (Liu et al., 2011). In this study, we synthesized the TiO_2 -GR composite using the AASA method without any reducing agents for the GR. The effects of the weight ratio of the GR/ TiO_2 in the colloidal mixture on the particle properties of the morphology and specific surface area were examined. In addition, the electrochemical properties of the as-prepared TiO_2 -GR composites for use in a glucose biosensor were evaluated by means of cyclic voltammetry measurements.

2. Experimental

2.1. Synthesis of the graphene oxide colloid

Graphene oxide (GO) was synthesized by the oxidation of graphite powder (Alfa Aesar, 99.9%) using H_2SO_4 (Junsei, 95%) and KMnO_4 (Junsei, 99.3%) according to the modified Hummers' method (Hummers and Offeman, 1958) with a pre-oxidation step, where the micrographic powder was mixed with a strong oxidizing agent and was then filtered, washed and dried. The dried GO was redispersed in water with mechanical agitation or mild sonication using a table-top ultrasonic cleaner, giving a colloidal solution of exfoliated GO, as noted in earlier reports (Cote et al., 2009a, 2009b; Hummers and Offeman, 1958; Kim et al., 2010a).

2.2. Synthesis of TiO_2 -GR composite

For the synthesis of the TiO_2 -GR composite, a colloidal mixture solution as an aerosol precursor was prepared by dispersing TiO_2 nanoparticles (P25, Degussa) and the as-prepared GO colloid into distilled water. The precursor was prepared with different weight ratios of GO/ TiO_2 from 0.05 to 2.0 while the concentration of the TiO_2 was fixed at 0.1 wt% in the colloidal mixture. The experimental apparatus for the AASA process consisted of an ultrasonic atomizer, an electrical tubular furnace, and a filter sampler. The ultrasonic atomizer was used to generate micron-sized droplets of the colloidal precursor. The droplets were then carried into the furnace by 1.0 l/min of argon. The evaporation of water, the self-assembly of GO and TiO_2 , and the reduction of GO were carried out in sequence in the tubular furnace, where the length and diameter of the heating zone were 54 cm and 25 mm, respectively, and the operating temperature was 800 °C. The fabricated TiO_2 -GR composites were then collected by a Teflon membrane filter. The filter was heated to prevent water from condensing on it. It took only several seconds to prepare the composite without the need for any post-heat-treatment or purification steps.

2.3. Synthesis of TiO_2 -GR/GOD electrode

1 mg of the as-prepared TiO_2 -GR composite was dispersed in 1 ml of 10,000 ppm (10 mg/ml) GOD solution (Sigma Aldrich, *Aspergillus niger*, 200 units/mg). The TiO_2 -GR/GOD colloid was stored at 4 °C for 24 h and separated by a centrifugal separator operating at 10,000 rpm for 1 h in order to ensure complete GOD immobilization. The precipitate was re-dispersed by 1 ml of 0.01 M PBS solution (Fluka). For the cyclic voltammetry measurement, 5 μl of TiO_2 -GR/GOD colloid was dropped onto the surface of a glassy carbon electrode (GCE) and left to dry at room temperature. Then, 10 μl of 0.05 wt% Nafion solution was additionally cast on the surface of the modified GCE and was dried before the electrochemical experiments. 0–8 mM of D-(+)-glucose (Sigma Aldrich) was used as the reaction solution.

2.4. Analysis

The particle morphology and size of the TiO_2 -GR composite were characterized by a field emission scanning electron microscope (FE-SEM; Sirion, FEI). The BET specific surface areas were measured by N_2 adsorption-desorption isotherms on a Quadrasorb Quantachrome analyzer. The crystallinity of the as-prepared composite was analyzed by X-ray diffractometer (XRD, Rigaku RTP 300 RC). X-ray photoelectron spectroscopy (XPS) measurement was carried out for an elemental analysis of the composite using a Sigma Probe photoelectron spectrometer (Thermo VG). The electrochemical properties of the glucose biosensor were measured by cyclic voltammetry (CV) method using an electrochemical interface instrument (VSP, Bio-Logics). A conventional three-electrode cell was used, including a glassy carbon electrode as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum foil as the counter electrode.

3. Result and discussion

3.1. Synthesis of TiO_2 -GR composite

Fig. 1 shows the X-ray photoemission spectroscopy (XPS) spectrum of the as-prepared GO showed that C and O peaks appeared at 285 and 533 eV, respectively. Also, C 1s spectrum shows two peaks at 284.7 and 286.6 eV, which are attributed to C–C and C–OH/C=O, respectively (Yang et al., 2009). According to

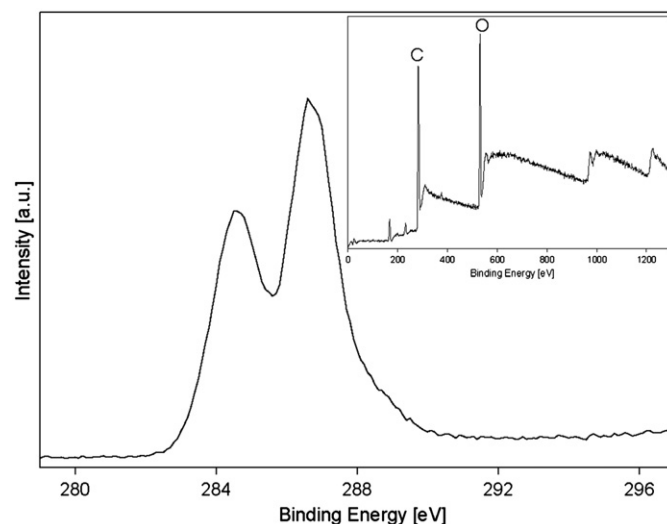


Fig. 1. X-Ray photoelectron spectroscopy of the as-prepared GO.

the result of the XRD analysis, the peak of GO appeared at 10.2° , which matched earlier results (Ishikawa et al., 2010). These results confirmed that GO was successfully synthesized from graphite by the modified Hummers' method.

In Fig. 2, the FE-SEM analysis exhibits the morphology of a TiO_2 -GR composite prepared at different ratios of GR/ TiO_2 . It was observed that the TiO_2 particles become encapsulated by the GR, as shown in Fig. 2(a). The formation of the TiO_2 -GR composite via the aerosol-assisted co-assembly of GO nanosheets and TiO_2 nanoparticles is explained as follows. As the aerosol droplets were introduced into the hot zone of the furnace, rapid

evaporation caused the shrinkage of the droplets, concentrating the TiO_2 nanoparticles and the GO sheets, and then subsequently compressing them into micron-sized composite (Jang et al., 2008; Jang et al., 2010; Luo et al., 2011). The GO sheets were also thermally reduced to chemically modified graphene. Because GO is effectively a 2D amphiphile with a hydrophilic periphery and a largely hydrophobic center, it was noted that GO sheets are surface-active with the ability to lower interfacial energies (Kim et al., 2010b; Cote et al., 2011). GO can accumulate at the water surface of a sprayed droplet during evaporation and finally form a GR ball. In contrast, hydrophilic TiO_2 nanoparticles agglomerate

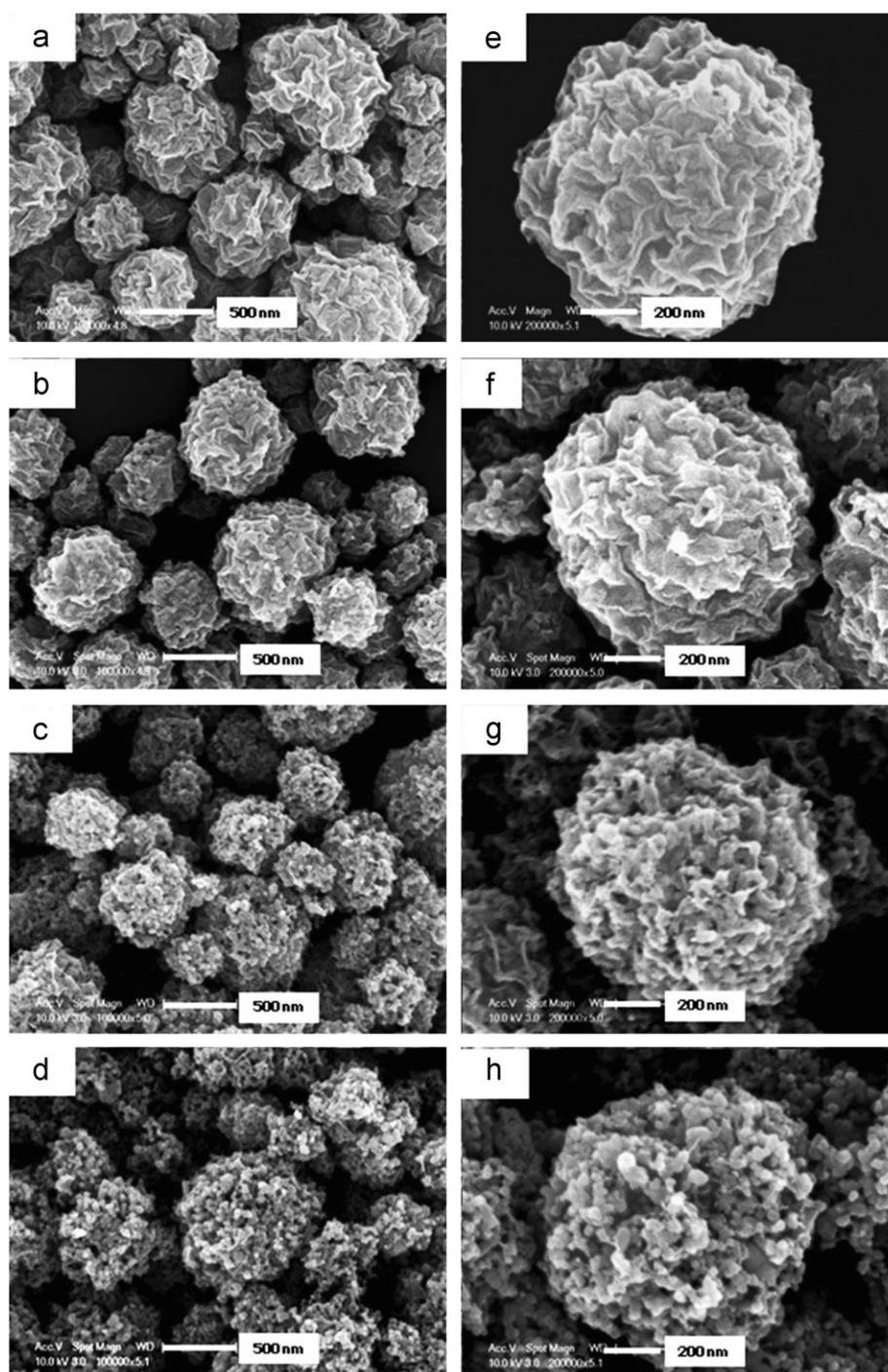


Fig. 2. FE-SEM images of TiO_2 -GR composite at different weight ratios of GO/ TiO_2 (a) 2, (b) 0.5, (c) 0.1, (d) 0.05, and (e,f,g,h) shows the magnified image of (a,b,c,d) at the fixed experimental condition (TiO_2 : 0.1 wt%, temp.: 800°C , carrier gas flowrate: 1 l/min).

and become positioned inside of the GR ball during the evaporation process. This explains why TiO_2 particles encapsulated by GR can be generated via the AASA technique. As the ratio of GO/ TiO_2 decreased from 2.0 to 0.05, a decrease in the degree of coverage of GR to TiO_2 was clearly observed.

The diffraction patterns of the TiO_2 -GR composite according to the XRD analysis show the anatase and rutile phase of TiO_2 only. Although the GR contents in the composite varied, the intensity of the TiO_2 phases did not change because the intensity of the GR phase was much lower than that of TiO_2 (Fig. S1). As the ratio of GO/ TiO_2 increased, the specific surface area of the TiO_2 -GR composite increased from 50 to $180 \text{ m}^2/\text{g}$ because the unique structure of GR has a high specific surface area (Table S1).

Fig. 3 shows the X-ray photoemission spectroscopy (XPS) spectra of TiO_2 -GR composites. The C, Ti and O peaks were measured at 285, 480 and 533 eV, respectively. As the GO/ TiO_2 ratio decreased, the intensity of C became lower whereas that of Ti and O became higher. This occurred because TiO_2 was exposed at a lower concentration of GR.

3.2. Evaluation of the electrocatalytic activity of glucose biosensors

Cyclic voltammetry was employed to measure the electrocatalytic activity of glucose biosensor for TiO_2 -GR composite. This method is a fast and convenient tool for characterizing glucose biosensor. In Fig. 4, characterizations of glucose biosensors prepared with GR, TiO_2 , and the TiO_2 -GR composite were done with 2 mM of glucose solution in each case. Among the biosensors based on the three samples, the biosensor prepared with the TiO_2 -GR composite showed the highest current flow as well as clear oxidation, with reduced peaks at -0.5 V and -0.6 V . In contrast, the current flow of the other samples did not show an obvious redox peak, which indicated that these electrodes were not electrochemically active. This is clear evidence that an electrochemical reaction occur on the TiO_2 -GR composite. It shows that the TiO_2 -GR composite demonstrates the superior ability of its enzyme, which results in the fast detection of glucose in terms of an electrochemical reaction.

Fig. 5 shows the cyclic voltammetry result of a glucose biosensor based on the TiO_2 -GR composite at different weight ratios of GO/ TiO_2 of 0.5, 0.1, and 0.05. Cyclic voltammetry of the TiO_2 -GR composite shows a strong dependence on the composite

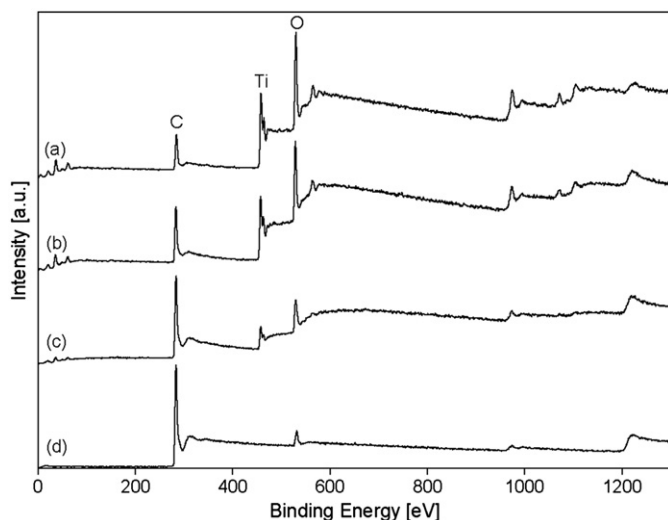


Fig. 3. X-ray photoelectron spectra of TiO_2 -GR composite prepared at different GO/ TiO_2 weight ratio (a) 0.5, (b) 0.1, (c) 0.05, and (d) pure GO 0.2 wt% at the experimental condition of Fig. 2 (TiO_2 : 0.1 wt%, temp.: 800°C , carrier gas flowrate: 1 l/min).

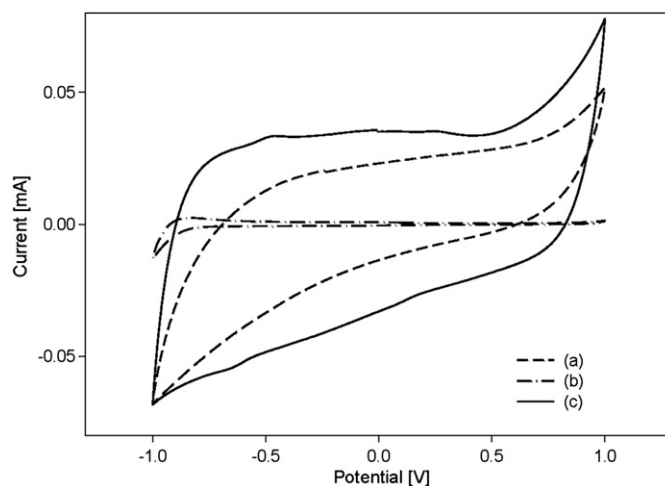


Fig. 4. Cyclic voltammograms of glucose biosensors prepared with GR, TiO_2 , and TiO_2 -GR composite, respectively (glucose conc.: 2 mM, potential range: -1.0 – 1.0 V , scan rate: 50 mV/s , (a) GO: 0.1 wt%, (b) TiO_2 : 0.1 wt%, (c) TiO_2 -GR composite: 0.05 wt% of GO/ TiO_2 (TiO_2 : 0.1 wt%)).

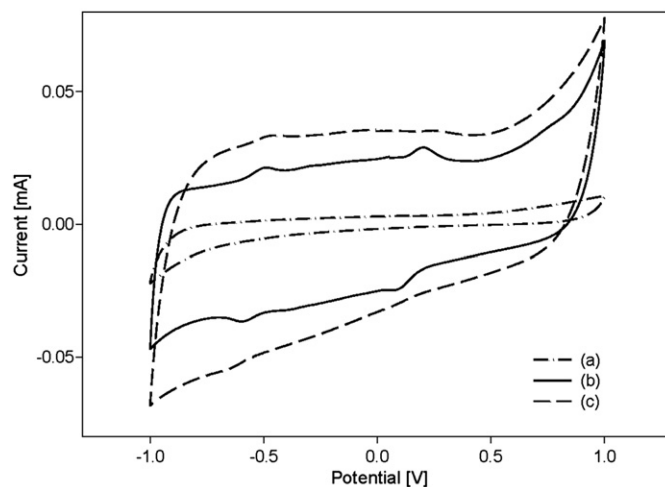


Fig. 5. Cyclic voltammograms of the glucose biosensor based on TiO_2 -GR composite prepared at different weight ratios of GO/ TiO_2 (a) 0.5, (b) 0.1, (c) 0.05 (TiO_2 : 0.1 wt%, glucose conc.: 2 mM, potential range: -1.0 – 1.0 V , scan rate: 50 mV/s).

morphology. The current flow of the biosensor prepared at a lower ratio of GR/ TiO_2 was stronger than that of a composite at a higher ratio of GR/ TiO_2 . In other words, the composite covered with less GR showed higher electrochemical activity, whereas thick layers of crumpled GR can obstruct the electron transfer. It indicates that TiO_2 -GR composite having large surface to volume ratio and high conductivity leads to enhanced enzyme absorption and promotes direct electron transfer between redox enzyme and the surface of electrode (Xu et al., 2010).

The stability of the glucose biosensor was measured with respect to the number of scanned cycle at 2–8 mM glucose solution using the TiO_2 -GR-GOD electrode. The cyclic voltammetry by the as prepared TiO_2 -GR-GOD electrode showed that response decrease in current was about 5.6, 5.4, 5.8% after the electrode was scanned continuously for 100th cycles at 2, 5, 8 mM glucose solution, respectively (Fig. S2). Therefore, we confirmed that the TiO_2 -GR-GOD electrode was stable and repeatable because the CV curves showed quite stable changed for 100th cycles.

Selectivity of the as-prepared TiO_2 -GR glucose biosensor was investigated in the presence of plasma samples such as ascorbic

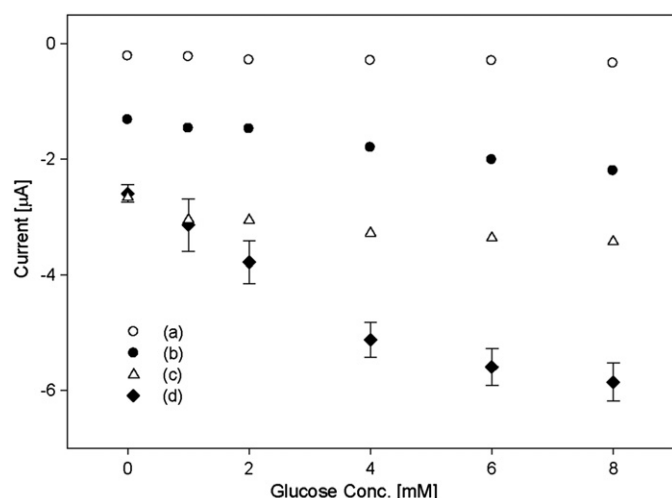


Fig. 6. Amperometric responses of the glucose biosensor based on $\text{TiO}_2\text{-GR}$ composite prepared at different weight ratios of GO/TiO_2 (b) 0.5, (d) 0.1, (a) pure TiO_2 0.1 wt%, and (c) pure GO 0.2 wt% (potential: -0.6 V, scan rate: 50 mV/s, error bar = $\pm$ standard deviation).

acid (AA) and uric acid (UA). We prepared 1 mM AA and UA solution, respectively. As those are minutely added into 20 ml PBS solution, there was no change in current after adding 1 ml of AA or UA, respectively. These results show that the as-prepared $\text{TiO}_2\text{-GR}$ glucose biosensor has the good selectivity for glucose.

The amperometric response by the glucose biosensor based on the $\text{TiO}_2\text{-GR}$ composite was linear against the concentration of glucose ranging from 0 to 8 mM at -0.6 V (Fig. 6). The relative standard deviation (RSD) of the current response to 8 mM glucose was 5.6% for 4 measurements, which assures stability of the $\text{TiO}_2\text{-GR}$ biosensor. The sensitivity of the $\text{TiO}_2\text{-GR}$ composite was determined by measuring the slope between glucose concentration and current. The highest sensitivity of the glucose biosensor based on the $\text{TiO}_2\text{-GR}$ composite was about $6.2 \mu\text{A}/\text{mM cm}^2$ when the composite was prepared at 0.1 of GR/TiO_2 . It is well known that the glucose concentration for a normal person ranges from 4.4 to 6.6 mM (Kuila et al., 2011). This indicates that the as-prepared $\text{TiO}_2\text{-GR}$ glucose biosensor is suitable for detecting the human blood sugar concentration for the diagnosis of diabetes mellitus. The response current of the glucose biosensor based on $\text{TiO}_2\text{-GR}$ was larger than those of the biosensors based on TiO_2 and GR. This suggests a synergistic effect of the electrocatalytic reaction for a glucose biosensor when TiO_2 nanoparticles are employed with GR as electrode materials due to the combination of the unique properties of GR and TiO_2 .

When we compared the electrochemical properties of the glucose biosensor based on $\text{TiO}_2\text{-GR}$ composite to those of previously reported sensors, our sensor shows the highest sensitivity and stability in the absence of novel metals (Table S2).

4. Conclusions

A spherical $\text{TiO}_2\text{-Graphene}$ composite was successfully prepared by rapidly evaporating droplets of a colloidal dispersion of GO nanosheets and TiO_2 nanoparticles via the aerosol assisted

self-assembly method. The $\text{TiO}_2\text{-GR}$ composite showed better catalytic performance for glucose redox than a GR or a TiO_2 electrocatalyst. In particular, the current flow of the composite which included the porous TiO_2 agglomerate partially covered with GR was stronger than that of the composite including the porous TiO_2 agglomerate fully covered with GR. This demonstrates the synergistic effect of using TiO_2 nanoparticles with GR as the electrode materials for biosensors. Therefore, the $\text{TiO}_2\text{-GR}$ composite shows promise as an enhanced glucose biosensor.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2012.05.033>.

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