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Graphene–gold nanoparticle composite: Application as a good scaffold for construction of glucose oxidase biosensor

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ABSTRACT

In the present work we report a facile method for fabrication of glucose oxidase immobilized on the partially reduced graphene–gold nanocomposite (PRGO–AuNPs/GOx) as a novel biosensor for determination of glucose concentration. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to study the morphology of PRGO and PRGO–AuNPs. Also, fast Fourier transformation infrared spectroscopy (FTIR) and UV–Vis spectroscopy were used to confirm formation of graphene and graphene–gold composite. Then, the electrochemical behavior of PRGO–AuNPs/GOx modified electrode was studied by cyclic voltammetry (CV). Our electrochemical studies, especially chronoamperometry (CA), showed that the PRGO–AuNPs/GOx modified electrode has excellent electrocatalytic activity towards the glucose. The limit of detection and sensitivity towards glucose were estimated as 0.06 μM and 15.04 mA mM^{-1}, respectively.

1. Introduction

Diagnosis of diabetes as its importance and universality needs great attention with precise monitoring of glucose level [1–3]. The typical method for monitoring glucose level from finger sticks may be painful, in addition to its relatively high cost, which needs more infection cares. Since the glucose level in the blood has linear dependency to its level in saliva, this may lead to an alternative non-invasive self-measurement of glucose level. Glucose level in human saliva for healthy body is in the range of 8–210 μM compared to blood glucose level (4–8 mM) [4,5]. Low glucose level (hypoglycemia) symptoms occur below this value (from 0.92 to 1.63 μM for salivary flow as average in diabetic patients [6]). Most of the existing biosensors work in milli-molar range, so micro-molar sensing of glucose level in biofluid with high sensitivity has attracted great importance [5,7]. Glucose enzymatic sensing based on glucose oxidase (GOx) has been commonly used due to its high sensitivity; selectivity and low detection limit [8–12]. There are several methods to determine the glucose concentration in which GOx catalyzes the reaction of glucose with oxygen and yield oxidized form of glucose. One of the most important methods to determine the glucose concentration includes oxygen measurement [13,14]. However, due to oxygen dissolution difficulties, detection of glucose concentration based on the detection of hydrogen peroxides (H_2O_2) as a product of the glucose oxidation reaction has been proposed. Hydrogen peroxide has relatively high overpotential against standard cells [15,16]. Probabilities of oxidation of the species during electrochemical measurements lead to comprehensive studies on electron transfer between enzyme and electrode surface. Effective immobilization of enzyme is a crucial issue to accomplish sufficient electrical communication between enzyme and electrode surface [17–22]. Various materials and methods have been developed to immobilize enzyme, including nanostructures like conducting polymers, carbon nanotubes (CNTs), graphene, metal and metal oxide nanoparticles [11,16,23–30]. It was observed that functionalized surface can affect the immobilization efficiency [31,32]. Recently, CNTs were investigated to immobilize the enzyme and the corresponding results confirm the importance of introducing appropriate functionalities [31,33]. Consequently, introducing a wide variety of functional groups especially carboxylic acid group has been studied [11,33,34]. Moreover, coupling of amino derivative group of glucose cofactor, Flavin Adenine Dincleotide (FAD), with carboxylic acid moiety has been well defined [10,11,28,33,34]. Of note, the enzyme active sites must be positioned efficiently in close vicinity to the electrode surface for effective electron transfer [31,34]. Also, it is proved that electrical conductivity and biocompatible microenvironment matrix is an essential need for biosensing purposes.

Graphene as an atom-thick two dimensional honeycomb carbon structure with high electrical conductivity, large active surface area, rapid electron transfer and good biocompatibility; has attracted great interests in bioelectrochemistry [8,22,25,35–37]. It was reported that the chemically reduced graphene oxide (RGO) may experience irreversible restacking because of van der Waals and πi–πi interactions, resulting in difficulties in processability and decrease in active surface area.

To prevent single graphene sheets from agglomeration in dry state, also addition of synergic effect of catalytic properties, conducting

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biocompatible nanostructures such as gold nanoparticles has been used [38,39]. Fabrication of Au nanoparticles (AuNPs) and graphene sheet composite is conducted through two main methods. The first method includes using an intermediate as linker between AuNPs and graphene [40–42]. Cao et al. have employed cationic polyelectrolyte protected AuNPs in order to immobilize glucose oxidase using a layer-by-layer method [43]. The decrease in conductivity of composite due to the presence of insulting materials has been reported as a major disadvantage in this method. The second method is in-situ synthesis of graphene–AuNP composite through co-reduction of graphene oxide and Au salt [44]. Cui and Zhang have fabricated graphene–AuNP composite to use in electrochemical sensor to determine the epinephrine concentration [40]. However, there are some limitations to the second method which are still challenging. The main challenges are difficulties to control the reduction process, homogeneous distribution of nanoparticles, and ability to control the morphology of AuNPs and the more important problem is lack of good processability. Low processability occurs since the resulting composite yield in the form of precipitate and biosensing applications requires dispersed materials [41,45]. It should be noted that intercalation of metal nanoparticles between reduced graphene oxide sheets may not happen in the abovementioned approaches. However, stability of graphene oxide in the presence of specific ionic strength is still under investigation [46–48].

Herein, we employed the optimum conditions of graphene reduction to maintain the conductivity of graphene sheets while keeping some of useful graphene oxide functionalities to ensure communication of enzyme with electrode surface. Keeping the functional groups helps to form stable colloid and provides homogeneous mixing of AuNPs and PRGO without any macromolecular additive. The stability of the colloids also improves processability and ease of casting of PRGO–AuNPs on glassy carbon electrode (GCE). In the present work, AuNPs and PRGO were synthesized separately by a well-defined method via $HAuCl_4$ reduction using sodium citrate. Condensation reaction between amino group moiety of enzyme and carboxylic group of PRGO enhances the kinetics of electron transfer.

2. Experimental

2.1. Materials

Glucose oxidase, hydrazine hydrate, and graphite powder were purchased from Sigma-Aldrich Company and used as received. Other chemicals were purchased from Merck Company and used without further purification. All solutions were prepared by using ultrapure water from a Millipore-MilliQ system. All the experiments were performed at ambient temperature.

2.2. Apparatus

All electrochemical experiments were carried out using an EG&G potentiostat–galvanostat (model 263-A, USA) equipped with Power Suite software package. Electrochemical studies were performed using a conventional three-electrode cell. A 3 mm modified GCE electrode, an Ag/AgCl and a platinum rod were used as working, reference and counter electrodes, respectively. All potentials were measured and reported against the Ag/AgCl reference electrode.

Scanning electron microscopy (SEM) was performed by Seron AIS2100, using 15 kV accelerating voltage. Transmission electron microscopy (TEM) was performed by a LEO 912AB electron microscope at 150 kV. Samples were prepared without gold coating due to sufficient conductivity. At first, samples were dried out at room temperature to avoid any excess reduction; then mixed with KBr to obtain test samples for FT-IR spectroscopy. The IR spectra were obtained from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} by a Nicolet-IR100 spectrometer. UV–Vis spectra were obtained using a Cecil CE9200, England.

2.3. Synthesis of GO and PRGO

GO was synthesized from graphite flake using modified Hummers method [49]. Briefly, 1 g of natural graphite powder and 0.5 g of $NaNO_3$ were added to 40 ml of H_2SO_4 solution and the resulting mixture was allowed to stir for 30 min (mild pre-oxidation step). Then, 4 g of $KMnO_4$ was added to this mixture and left it at 30 °C for 120 min, to form a paste. In the next step, 200 ml of water and 17 ml of H_2O_2 solution (30 wt.%) were added to the mixture to obtain graphite oxide mixture. The graphite oxide mixture was then centrifuged and washed three times with a 1.0 M HCl solution and water. Finally, the resulting solution was sonicated (CD-4820 40W) for 30 min to produce graphene oxide dispersion.

In order to yield stable dispersion of partially reduced graphene oxide (PRGO), chemical reduction with hydrazine was applied through the procedures suggested by Li and co-workers [50]. Briefly, GO dispersion was diluted to 0.5 mg/ml. Then, 85 ml of as-prepared dispersion was mixed with 0.06 ml of hydrazine (55% wt in water). The weight ratio of hydrazine to GO was controlled at 0.7. Also, pH was adjusted with adding appropriate amount of ammonia solution (28 wt.% in water) to convert carboxylic acid group to the corresponding carboxylate form. The mixture was stirred using a magnetic stirrer for 10 min, then it was put in a 95 °C water bath for 1 h. The stable PRGO dispersion was yielded after removing the black precipitates via glass cotton filtration. Scheme 1 displays the GO and PRGO synthesize route.

2.4. Synthesis of AuNPs and PRGO–AuNPs

Colloidal AuNPs were prepared according to the literature by reaction of sodium citrate solution and $HAuCl_4$ solution which was previously heated up to 60 °C. Briefly, 0.5 ml of 1% (w/v) sodium citrate solution as reducing agent was added dropwise to 50 ml of 0.01% (w/v) $HAuCl_4 \cdot 3H_2O$. The resulting mixture was boiled for 15 min. Then, the prepared stable colloid was stored in dark glass bottle at 4 °C. Additionally, stable dispersion of AuNPs was added to PRGO colloid with concentration of 0.5 mg ml^{-1} and mixed in a shaker for overnight to ensure the complete intercalation of nanoparticles between PRGO sheets. The weight ratio of AuNPs to PRGO was adjusted at 1. Scheme 2 is a simple representation of the applied strategy for preparation of PGRO–AuNPs.

2.5. Preparation of PRGO–AuNPs–GOx–GCE

At first, a GC electrode was polished with 0.5 and 0.05 μm Al_2O_3 powder to reach to the mirror finish surface. Then, it was rinsed with water and sonicated in ethanol and doubly distilled water. The cleansed GCE was gently dried under a nitrogen atmosphere. In the next step, 5 μl of PRGO–AuNP composite (1 mg ml^{-1}) was dropcasted on GCE, and then it was left in room temperature to evaporate its solvent. Finally, 2 μl of GOx solution (10 mg ml^{-1}) was dropcasted on the PRGO–AuNP modified electrode. The fabricated PRGO–AuNPs–GOx electrode was rinsed with ultrapure water to wipe off any loosely attached GOx. In a control experiment, to check the effects of AuNPs, the similar procedure was repeated to modify a GCE in which pure PGRO was dropcasted on the electrode. These two modified electrodes were denoted as PRGO–AuNPs–GOx and PRGO–GOx, respectively. The voltammetric behavior of PRGO–AuNPs–GOx and PRGO–GOx modified electrodes was investigated at various pH (from 5.8 to 8) by cyclic voltammetry at a scan rate of 100 mV s^{-1} in the air saturated condition. To determine the glucose concentration, electrochemical tests were performed in 0.1 M of phosphate buffer solution (PBS). Scheme 3 is a graphical representation of the abovementioned procedures.