



Green synthesis of reduced graphene oxide decorated with gold nanoparticles and its glucose sensing application



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ARTICLE INFO

Article history:

Received 15 April 2014

Received in revised form 23 May 2014

Accepted 23 May 2014

Available online 2 June 2014

Keywords:

Green chemistry

Rose water

Reduced graphene oxide decorated with gold nanoparticles

Glucose oxidase

Biosensor

ABSTRACT

Here, we report an eco-friendly method for synthesis of reduced graphene oxide decorated with gold nanoparticles (rGO-Au_{nano}) by using rose water as reducing agent. The prepared materials were characterized using UV–visible absorption spectroscopy, Raman spectroscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM) and X-ray diffraction (XRD). Furthermore, the direct electrochemistry of glucose oxidase (GOD) was achieved at a glassy carbon electrode modified with rGO-Au_{nano}. The resulting biosensor exhibited good response to glucose with linear range from 1 to 8 mM with a low detection limit of 10 μM.

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1. Introduction

Diabetes is a major public health problem in the worldwide which classed as a metabolism disorder. A new report has established that there are now nearly 350 million people on earth who suffer from diabetes [1]. Therefore, the determination and controlling of blood glucose is very important, which if not controlled, it can cause retinopathy, nephropathy, neuropathy, hypertension, heart disease, stroke, gastroparesis, peripheral arterial disease, cellulitis and depression [2]. Up to now, several analytical methods, such as UV fluorescence [3,4] chemiluminescence [5] and titrimetry [6] have been proposed for the determination of glucose. However, these methods are generally time-consuming, lack of sensitivity, susceptibility to interference by other substances in analyte samples and difficult for an automated detection. To overcome all these shortcomings, the electrochemical biosensor based on the direct electrochemistry between an electrode and the immobilized glucose oxidase is especially promising because of its simplicity, high sensitivity and selectivity [7,8]. Recently, several materials for the fabrication of glucose biosensor based on direct electron transfer have been reported in the literature [7,9–12]. Among them,

the carbon based nanomaterials such as graphene nanosheet provides not only a large microscopic surface area Immobilization of enzymes, but also it provide a desirable biocompatible microenvironment [13–17]. Graphene, a single aromatic sheet of sp² bonded carbon, has been shown to possess unique electronic, optical, thermal, mechanical and catalytic properties which are attractive for widely varied potential applications in many fields of science ranging from nanoelectronics to biomedical devices [18,19]. This nanosheet can be synthesized by various methods [20]. Among them, the chemical reduction of exfoliated GO with green reducing agents have become a favorable topic for researchers. In recent years, increasing numbers of researches in field of nanoscience have been also devoted to green synthesis of reduced graphene oxide (rGO) decorated with novel metal nanoparticles such as rGO/Pt [21], rGO/Ag [22] and rGO/Au [23], opening the door to application in nanobiotechnology. The decoration of graphene with nanoparticles might be led to new composite with different properties via cooperative interaction. In this paper, we report a green method for synthesis of reduced graphene oxide decorated with gold nanoparticles (rGO-Au_{nano}) by using rose water as reducing agent. Also, we show that the prepared modified electrode with rGO-Au_{nano} nanocomposite would enhance the direct electron transfer capability of glucose oxidase (GOD) on the electrode surfaces. The prepared biosensor exhibited high sensitivity, anti-fouling properties, lower detection limit and high stability for glucose sensing.

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2. Experimental

2.1. Materials

All chemicals were of analytical reagent grade and used without further purification. Double distilled water was used throughout. GOD from *Aspergillus niger*, 221 U mg^{-1}) and D-(+)-glucose (97%) were obtained from Sigma (St. Louis, MO, USA). 10 mg of GOD was dissolved in 1 mL of phosphate buffer solution (0.05 M, pH 7.0) to prepare GOD working solution. A stock solution of glucose (0.5 M) was prepared with doubly distilled water and stored at 4°C when not in use. The glucose stock solution was allowed to mutarotate at room temperature for at least 24 h before use. Rose water (12 mg natural essence per 100 mL (so called 12%), Rabee Golab, Iran) was obtained from a local market.

2.2. Apparatus

UV-visible absorption spectra were recorded using a single beam Pharmacia UV-vis spectrophotometer (Ultrospec, model 4000). Raman scattering was performed on a Almega Thermo Nicolet Dispersive Raman spectrometer using the second harmonic (532 nm) of a Nd:YLF laser source. Scanning electron microscopy (SEM) was performed with a Philips instrument, Model XL-30. Atomic force microscopy (AFM) was conducted with a DME DualScope Scanner DS 95-200. X-ray diffraction (XRD) was performed with a D8ADVANCE (Bruker) X-ray diffractometer equipped with a $\text{Cu K}\alpha$ (1.5406 Å) radiation source. Cyclic voltammetry (CV) was performed using an Autolab potentiostat-galvanostat model PGSTAT30 with a conventional three-electrode setup, in which a bare glassy carbon (GC) electrode or modified GC electrode, an $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{sat}}$ and a platinum rod served as the working, reference and auxiliary electrodes, respectively. Electrochemical impedance spectroscopy (EIS) experiments were carried out using a Zahner Zennium workstation in the presence of $5.0 \text{ mM Fe}(\text{CN})_6^{3-/4-}$ couple (1:1) as the redox probe. The oscillation potential was 5 mV and the applied potential was 0.23 V. Also, a frequency range of 100 kHz to 0.1 Hz was applied and the output signal was acquired with the Thales z (Zennium release) software.

2.3. Preparation of the rGO-Au_{nano} solution

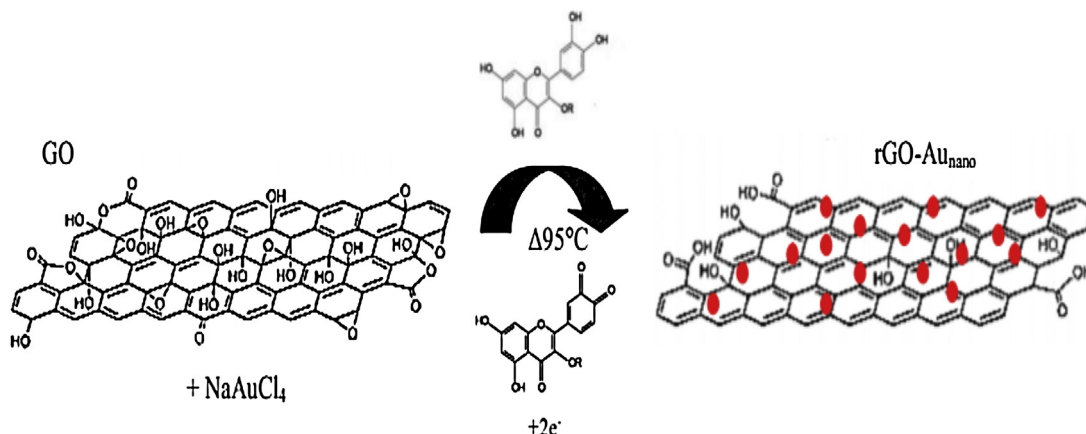
Graphite oxide was synthesized using graphite powders by the modified Hummers method [24]. Briefly, 5.0 g graphite powder was dispersed in a 120 mL concentrated H_2SO_4 kept at 0°C under stirring. Then, 15.0 g of KMnO_4 was added gradually to the mixture kept in an ice bath to ensure that the temperature remained around

0°C . After that, the temperature was raised to 0°C and the mixture was stirred for 30 min and then diluted gradually with 225 mL deionized water. The mixture was re-diluted with 700 mL deionized water and treated with 3% hydrogen peroxide. The color of mixture changed to yellow-brown during the drop wise addition of H_2O_2 . The mixture was filtered and washed with HCl solution (5%) and then repeatedly washed with water until neutral pH was obtained for filtrate. This solution was centrifuged at 3000 rpm for 10 min and then the filtrate was re-dispersed in water and centrifuged for several times. Finally, the dark brown GO powder was obtained through drying at 50°C in a vacuum oven for a day. The graphite oxide was then exfoliated by ultrasonication. For this purpose, GO powder dispersed in a known volume of water was subjected to ultrasonication for 60 min to give a stable suspension of GO and then centrifuged at 3000 rpm for 30 min to remove any aggregates remained in the transparent light brown exfoliated GO suspension. Then, 10 mL of rose water and $100 \mu\text{L NaAuCl}_4$ (0.01 mM) were added into 10 mL above solution and the solution was mixed by ultrasonication for 30 min. The mixture was transferred into a Teflon-lined stainless steel autoclave and reacted at 95°C for 5 h. The resulting rGO-Au_{nano} suspension was filtered and washed with plenty of water. The Scheme 1 illustrates the mechanism of rGO-Au_{nano} synthesis by phenolic compounds in rose water. The proposed mechanism is based on the reducing capability of phenolic compounds that contained in rose water [25].

3. Results and discussion

3.1. Characterization of the composites

We monitored UV-visible absorption spectroscopy GO and rGO-Au_{nano}, firstly. As shown in Fig. 1A, the absorption peak at 230 nm shifted to 263 nm, indicating that the electronic conjugation within the reduced graphene sheet was revived upon reduction of graphene oxide [26,27]. Also, the absorption was obtained at wavelength 530 nm showing the formation of gold nanoparticles. Raman spectroscopy is a powerful nondestructive tool to distinguish ordered and disordered crystal structures of carbon. G band is usually assigned to the E_{2g} phonon of C sp^2 atoms, while D band is a breathing mode of κ -point phonons of A_{1g} symmetry [28,29]. The Raman spectrum of the prepared GO, rGO and rGO-Au_{nano} showed two absorption bands (for D and G bands, respectively) (Fig. 1B). The increase of D/G intensity ratio is predicted for rGO after reduction of the GO because of the restoration of sp^2 domain [30–32], contrary to that observed in Fig. 1B. Also, Compared with D/G intensity ratio (1.24) of rGO, the D/G intensity ratio (1.05) of rGO-Au_{nano}, which indicates that Au nanoparticles aggregated on rGO can decrease the



Scheme 1. The mechanism of rGO-Au_{nano} synthesis.

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