Investigation of the optimal weight contents of reduced graphene oxide–gold nanoparticles composites and theirs application in electrochemical biosensors

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ABSTRACT

Graphene, as a single-atom-thick carbon material, is considered an ideal platform for designing composite nanomaterials for high-performance electrochemical or electrocatalytic devices. Reduced graphene oxide–gold nanoparticles composites were prepared by depositing gold nanoparticles (AuNPs) on the surface of reduced graphene oxide (RGO) with different RGO-to-AuNPs weight ratios. The resulting composite materials were characterized morphologically and optically by scanning electron microscopy (SEM) and UV–visible absorption spectroscopy. Cyclic voltammetry and amperometric measurements were employed to investigate the electrocatalytic effect of different composites toward the reduction of hydrogen peroxide. Experimental results demonstrated that RGO–AuNPs composites displayed high stability and catalytic effect for the analysis of hydrogen peroxide, demonstrating the possible synergistic effects of the RGO–AuNPs composite materials. Additionally, direct electron transfer of glucose oxidase (GOD) was achieved after codeposition of GOD and chitosan (CHIT). The glassy carbon electrode modified with RGO–AuNPs/CHIT–GOD material exhibited an excellent catalytic effect for glucose detection with a sensitivity of 34 mA M^{-1} cm^{-2} at a detection potential of -0.3 V vs. Ag|AgCl reference.

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1. Introduction

Graphene has attracted enormous interest because of the excellent properties, such as high surface area, superior electric conductivity and good mechanical strength [1,2]. The special two-dimensional (2-D) single-atom-thick structure of graphene also plays an important role in the fabrication of materials with different spatial structures. Graphene is also recognized as the basic building block of carbon materials [3,4] and has been utilized in the development of energy-storage materials [5,6], liquid crystal devices [7], polymer composites [8] and electrochemical applications [9,10]. However, the irreversible agglomerates of graphene are readily formed due to the van der Waals interactions [11]. In order to reduce the aggregation, some molecules and polymers such as polyvinylpyrrolidone [12], octadecylamine [13] have been used to modify and protect graphene. The hybridization of graphene with some inorganic particles has also been recognized as an ideal method for reducing the aggregation of graphene, because of the formation of some new graphene-based nanocomposites

[11]. Some researches demonstrated that graphene favoured the dispersion and stabilization of metal nanoparticles, such as Pt, Au and Pd [11,14].

On the other hand, gold nanoparticles (AuNPs) offered many applications in biosensor fabrication and enhanced analytical performance because they provided a suitable microenvironment for retaining the biomolecule activity and allowed direct electron transfer between redox proteins and electrode surface without redox mediators [15]. The high surface area and good electron conductivity of AuNPs facilitated electron transfer and served as a suitable candidate for biosensor fabrication [16].

Recently, many electrochemical biosensors based on the composites of nanomaterials have been developed, including polymer–graphene composites [17,18], redox mediators with carbon nanotubes [19,20] and metal particles–graphene composites [21,22]. Nanomaterial composites frequently offered improved electron transfer characteristics. Moreover, graphene (or reduced graphene oxide (RGO)), usually produced by the reduction of graphene oxide, possesses some characteristic functional groups such as hydroxyl (–OH) and carboxyl (–COOH) groups [23]. These functional groups were usually utilized as active sites for the nucleation and growth of metal nanoparticles in order to prepare RGO-based composites [24]. Composites of AuNPs and graphene

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have been synthesized by various methods such as in situ growth [14,24] and electrochemical deposition [25], and the application of the composites in the fabrication of electrochemical sensors have been reported [26,27]. Composite nanomaterials offered improved electron transfer characteristics resulted from possible synergistic effect [19,26,28]. Many reports demonstrated that RGO–AuNPs composites with good dispersity can be obtained through controlling the loading amount of the AuNPs on the graphene surface [24,27]. However, only few researches focus on the impact of weight ratio of RGO and AuNPs on the properties of composites obtained, such as the particles size, dispersity, optical and electrochemical properties.

In this work, AuNPs were prepared through the reduction of $HAuCl_4$ by sodium citrate. Reduced graphene oxide–gold nanoparticles (RGO–AuNPs) composites were obtained through nucleation and growth of gold particles on the surface of reduced graphene oxide. The optimal weight contents of RGO and AuNPs in the formation of RGO–AuNPs composites was investigated and characterized by SEM, ultraviolet–visible spectroscopy and electrochemical techniques. The RGO–AuNPs composites displayed excellent catalytic activity toward the reduction of H_2O_2, attributing to the synergistic effects between RGO and AuNPs. Additionally, glucose oxidase (GOD) was immobilized on the GC/RGO–AuNPs surface through electrostatic interactions between chitosan coating and RGO–AuNPs materials. The resulting sensor achieved direct electron transfer of GOD and was applied for the detection of glucose. The sensitivity for glucose detection was roughly two times higher than that reported by others for similar electrode system [24].

2. Experimental

2.1. Reagents and apparatus

Reduced graphene oxide (RGO) was purchased from XF Nano Material Co., Ltd. (Nanjing, China). Glucose oxidase (GOD) (Type X-S, from Aspergillus niger), β-D-glucose, chitosan (CHIT), dopamine hydrochloride (DA), uric acid (UA), gold(III) chloride trihydrate ($HAuCl_4·3H_2O$) were obtained from Sigma. Citric acid trisodium salt dihydrate and ascorbic acid (AA) were obtained from Aldrich. Hydrogen peroxide (H_2O_2) and potassium hexacyanoferrate ($K_3Fe(CN)_6$) were purchased from International Laboratory. All other reagents were of reagent grade and were used without further purification. Phosphate buffer solution (pH 7.4) consisting of 0.02 M NaH_2PO_4 and 0.02 M Na_2HPO_4 was used as the supporting electrolyte. All solutions were prepared with deionized water.

The morphologies and surface structures were characterized by a scanning electron microscope (SEM) (LEO, Electron Microscopy Inc., Cambridge, UK) operated at 20 kV. The SEM samples were prepared by placing 5 μL sample dispersion onto a glassy carbon substrate, and allowing them to dry in air. Ultraviolet–visible absorption spectra were recorded using a Cary 100-Scan UV–visible spectrophotometer (Varian Inc., USA). All electrochemical experiments were carried out at room temperature using a single compartment, three-electrode cell with the modified electrode as working electrode, a platinum wire and Ag|AgCl as auxiliary and reference electrodes, respectively. All potential were measured and reported vs. the Ag|AgCl reference electrode (sat. KCl). Cyclic voltammetry and amperometric measurements were performed on a CHI6012B electrochemical workstation (CH instruments, Inc., USA).

2.2. Preparation of RGO–AuNPs composite materials

RGO–AuNPs composite solution was prepared according to Natan [29]. Typically, 0.2 mL $HAuCl_4$ (20 mg/mL) and 0.1 mL RGO (2 mg/mL) solutions were added to 20 mL of boiling deionized water with vigorous stirring. Then, 0.8 mL sodium citrate solution (10 mg/mL) was rapidly added to the RGO–$HAuCl_4$ mixture. The resulting mixture was stirred and heated for 15 min with a color change from pale yellow to pink. The resulting mixture was immediately collected by centrifugation and thoroughly washed with deionized water. The resulting product was re-dispersed in 0.2 mL deionized water under sonication to produce a colloidal suspension (designated as Composite B in this study). A series of RGO–AuNPs composites with various weight ratios of RGO and AuNPs were prepared. The weight ratios of the composite mixtures can be easily controlled by changing the volume of precursor materials (RGO, $HAuCl_4$ and sodium citrate) employed for preparation. The compositions of mixtures employed for the preparation of different RGO–AuNPs composites are listed in Table 1 (assuming complete conversion of $HAuCl_4$ to AuNPs). In addition, pure AuNPs were synthesized similarly without the addition of RGO.

2.3. Preparation of modified electrodes

Glassy carbon electrodes (GCE, Bioanalytical Systems, Inc., 3 mm in diameter) were carefully polished with 0.3 and 0.05 μm alumina slurry on a microcloth (Buehler, USA), followed by ultrasonication in ethanol and deionized water for 2 min. Modified electrodes were prepared by adding 5 μL of the target suspension (RGO, AuNPs or RGO–AuNPs composites) on the GC electrode surface and were allowed to dry in air.

For the preparation of glucose biosensors, 5 μL 3% chitosan (CHIT) solution containing 10 mg/mL glucose oxidase (GOD) was cast onto the surface of GCE/RGO–AuNPs modified electrode and was allowed to dry at room temperature. The biosensors were stored at 4 °C before use.

The steady state amperometric response to H_2O_2 and glucose were measured by voltammetric technique in a degassed 0.02 M phosphate buffer solution (PBS, pH 7.4) under gentle stirring of around 100 rpm at the desired potentials. The current response was recorded on successive addition of the substrates.

3. Results and discussion

3.1. Effects of preparation composition

The deposition of the gold nanoparticles (AuNPs) on the surface of reduced graphene oxide (RGO) produced a 3-D structure, which greatly enhanced the surface area and offered good electrochemical properties. On the other hand, the size, density and morphology of the resulting reduced graphene oxide–gold nanoparticles (RGO–AuNPs) composites changed with the different weight ratio of RGO and AuNPs employed in the preparation. To explore the effects of preparation compositions on the physical and chemical properties of the RGO–AuNPs composites, different RGO–AuNPs composites were synthesized (as detailed in Table 1) and various techniques (SEM, UV–visible and electrochemical techniques) were employed for characterization. Fig. 1 shows the SEM images of RGO–AuNPs composites prepared with different preparation compositions (RGO, $HAuCl_4$ and sodium citrate). The amount of sodium citrate was in excess and should be enough to convert all $HAuCl_4$ reagents to give AuNPs. RGO materials gave a flake-like thin wrinkling film structure, as shown in Fig. 1(h), similar to those reported in the literature [12,30]. On the other hand, gold nanoparticles were well dispersed on the glassy carbon surface, as shown in Fig. 1(i). The average size of AuNPs was about 26 nm.

RGO–AuNPs composites prepared with different compositions displayed very different morphologies, as evidenced from the SEM images shown in Fig. 1(a)–(g). For all the composites prepared