



# Nitrite electrochemical biosensing based on coupled graphene and gold nanoparticles



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## ABSTRACT

Biofunctionalized graphene-gold nanoparticle (AuNP) hybrids were prepared using a facile approach of *in situ* growth, with homogeneous distribution of AuNPs on the graphene nanosheets. Hemoglobin (Hb) was immobilized on the graphene-AuNP composites to fabricate biosensors for determination of nitrite ( $\text{NO}_2^-$ ). A pair of well-defined redox peaks was observed for Hb immobilized on the graphene-AuNP hybrids with a formal potential ( $E^0$ ) of  $-0.314$  V in 0.1 M phosphate buffered saline (0.15 M NaCl, pH 7.0). The novel biosensors exhibited many advantages, such as wide linear response range (from 0.05 to 1000  $\mu\text{M}$ ,  $R^2=0.997$ ), low detection limit (0.01  $\mu\text{M}$ , a signal to noise ratio of 3), high sensitivity (0.15  $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$ ), and excellent selectivity. These constructed biosensors were further used for determination of nitrite in pickled radish. The results obtained were in good agreement with those using spectrophotometry based on the National Food Safety Standard (GB 5009.33-2010), which indicates that these novel and sensitive biosensors have promising application for determination of nitrite in food.

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## 1. Introduction

Graphene (GA), a two-dimensional single-atom-thick conjugated carbon network, has attracted tremendous attention recently (Allen et al., 2010), because of its extraordinary properties, such as excellent electronic conductivity, large specific surface area, strong mechanical strength, and enhanced electrocatalytic activity. The unique nanostructure and properties hold great promise for potential applications in a wide variety of technological fields, for instance, nanoelectronics, supercapacitors, batteries, nanocomposites, and sensors. Up to now, graphene has been synthesized by micromechanical cleavage (Novoselov et al., 2004), chemical vapor deposition (Kim et al., 2009), epitaxial growth (Berger et al., 2006), liquid-phase exfoliation (Hernandez et al., 2008), and chemical reduction of graphene oxide (GO) (Li et al., 2008). However, the reduced species of GO tend to generate irreversible agglomerates and even restack to develop graphite, because of  $\pi$ - $\pi$  stacking interactions and highly cohesive van der Waals energy (Williams et al., 2008). Thus, chemical modifications of graphene become essential to improve its stability and introduce special functionalities. It is reported that aggregation of graphene nanosheets could be minimized or even inhibited by the formation of graphene-metal nanoparticle composites (Si and Samulski, 2008).

Many nanomaterials, such as Au (Goncalves et al., 2009), Pt (Guo et al., 2010), Pd (Sundaram et al., 2008),  $\text{TiO}_2$  (Williams et al., 2008), tin oxide (Paek et al., 2009), latex (Liu et al., 2010), and carbon nanotube (CNT) (Tung et al., 2009), were used to fabricate functional graphene nanocomposites. Among them, gold nanoparticles (AuNPs) with unique properties provide suitable microenvironments for immobilization of biomacromolecules (Shan et al., 2010) and allow for direct electron transfer between redox-active proteins and bulk electrode materials (Pingarrón et al., 2008); thus AuNPs are widely used nanomaterials for the fabrication of electrochemical biosensors. So far, two main methods have been developed to construct graphene-AuNP hybrids. One is using media, such as bovine serum albumin, which act as both stabilizers and crosslinkers for preparation of graphene-medium-AuNP composites (Hong et al., 2010). However, the obstacle of nonconducting media between graphene and AuNPs gives rise to poor electroconductivity of the hybrids. In order to solve out the problem, the method for adhesion of metal nanoparticles to graphene has been proposed. Nevertheless, the method for assembly of AuNPs on graphene has three primary limitations (Liu et al., 2010): (i) good control over reaction process is lacking; (ii) resulting nanocomposites are mostly in the form of precipitates instead of well-dispersed nanomaterials; (iii) homogeneous distribution of AuNPs on graphene nanosheets without “glue” remains challenging. Herein, we report a facile method for *in situ* growth of AuNPs on graphene nanosheets. The loading amount of AuNPs formed could be easily controlled by adjusting the mass ratio of graphene and  $\text{HAuCl}_4$ .

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The determination of nitrite ( $\text{NO}_2^-$ ) is of great importance since nitrite is widely used in the environment, beverages, and food products as a preservative (Ding et al., 2010). Moreover, nitrite can become poisonous at high concentrations to animals and human beings because it probably results in generation of carcinogenic nitrosamines (Lijinsky and Epstein, 1970). A variety of techniques have been developed to detect nitrite, such as spectrophotometry (Kuznetsov and Zemyatova, 2007), chromatography (Niedzielski et al., 2006), capillary electrophoresis (Kikura-Hanajiri et al., 2002), chemiluminescence (Lagalante and Greenbacker, 2007), and electrochemistry (Yue et al., 2011). Among them, electrochemical techniques are preferred because of their rapid response, convenient use, high sensitivity, good selectivity, time saving, and low cost. Generally, the electrochemical methods are based on either oxidation or reduction of nitrite at different electrodes (Wu et al., 2013; Wang et al., 2013; Jebaraj et al., 2012). Anodic oxidation of nitrite is usually preferred with the final product of nitrate ( $\text{NO}_3^-$ ), since main limitations in cathodic reduction of nitrite such as the interferences from several products depending on electrode condition and nature of catalysts employed can be avoided (Heinecke et al., 2013). However, few studies for oxidation of nitrite have been reported owing to the relatively high overpotential (Rajesh et al., 2010). Therefore, we attempt to prepare chemically modified electrodes for oxidation of nitrite with a low detection limit.

Herein, a facile approach was used to prepare stable aqueous dispersions of graphene with biocompatible chitosan (GACS). AuNPs were reduced *in situ* on the surface of graphene (Au/GACS) in the presence of sodium citrate. Hemoglobin (Hb)-immobilized Au/GACS (Hb/Au/GACS)-modified glassy carbon electrodes (GCEs) were used for the electrochemical detection of nitrite in high sensitivity and wide concentration range. To the best of our knowledge, no work has been reported about oxidation of nitrite using graphene relevant biosensors with a rather low detection limit of nitrite (as low as 0.01  $\mu\text{M}$ ). The electrochemical biosensors were used for practical application in determination of nitrite in pickled radish, and the results obtained were consistent with those by UV-vis spectrophotometry based on the National Food Safety Standard (GB 5009.33-2010).

## 2. Experimental section

### 2.1. Chemicals and materials

Graphite powder and acetic acid were purchased from Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium nitrite ( $\text{NaNO}_2$ ) was purchased from Shanghai Sinpeuo Fine Chemical Co., Ltd. Hydrazine hydrate (85 wt%), chloroauric acid ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ), trisodium citrate, and inorganic salts ( $\text{NaNO}_3$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{Na}_3\text{PO}_4$ ,  $\text{Na}_2\text{SO}_3$ ,  $\text{Na}_2\text{S}_2\text{O}_3$ , KBr, KI, and  $\text{AgNO}_3$ ) were obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Hb from bovine blood was purchased from Sigma-Aldrich. Chitosan (CS, deacetylation > 90.0%) from Shanghai Lanji Technology Co., Ltd. was used, and 2% (w/v) CS solution was prepared by dissolving CS in 1% acetic acid solution (v/v) under magnetic stirring for 2 h. Phosphate buffered salines (PBS, 0.1 M) containing 0.15 M NaCl at different pH were prepared by mixing different proportions of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ . All other chemicals were of analytical grade and used without further purification. Double-distilled water was used for preparation of aqueous solution.

### 2.2. Synthesis of graphene

GO was prepared from graphite via the Hummers and Offeman (1958) method. GACS was prepared by chemical reduction of GO similar to the method reported recently (Wu et al., 2010).

The as-synthesized GO was suspended in double-distilled water to produce an aqueous solution of 0.05 wt%. Then, 5 mL of the solution was added to 1.56 mL of 2% (w/v) CS, and the solution mixture was ultrasonicated for 30 min to give a light yellow solution. After that, 40  $\mu\text{L}$  of hydrazine hydrate (85 wt%) was added to the light yellow dispersion. The mixture was stirred for 5 min followed by heating at 100 °C for 24 h. The solution mixture underwent a change in color from light yellow to black, indicative of the reduction of GO. The black aqueous GACS dispersion was stable over one month at room temperature.

### 2.3. Preparation of graphene-AuNP hybrids

The preparation of graphene-AuNP hybrids is illustrated in Fig. 1A. First, 0.5 mL of 1 wt% aqueous  $\text{HAuCl}_4$  solution was added dropwise to 5 mL of the above GACS dispersion. The mixture was incubated for 30 min to promote the formation of gold nuclei from the reduction of  $\text{HAuCl}_4$  in the presence of hydrazine. Then, the solution was stirred at 60 °C for 2 h, after that, 1.0 mL of 0.1 M freshly prepared sodium citrate was added dropwise. Finally, the reaction was kept under the conditions for 1 h. For morphological analysis, the resulting precipitates were rinsed with double-distilled water by centrifugation to remove unbound AuNPs in the solution.

### 2.4. Preparation of Hb/Au/GACS/GCE biosensors

As illustrated in Fig. 1B, multistep processes were included in the fabrication of an electrochemical sensor. First, the surface of a GCE (3 mm in diameter) was polished sequentially with 1.0, 0.3, and 0.05  $\mu\text{m}$  of alumina slurry, followed by rinsing thoroughly with double-distilled water. The electrode was successively washed under ultrasonication in 1:1 nitric acid, acetone, and double-distilled water followed by drying with nitrogen. Second, 5  $\mu\text{L}$  of the graphene-AuNP hybrids were dropped by a pipette onto the pretreated GCE surface (noted as Au/GACS/GCE) and allowed to dry in a vacuum desiccator for 2 h. Finally, the graphene-AuNP modified electrode was cast with an aliquot of 5  $\mu\text{L}$  of Hb (10 mg  $\text{mL}^{-1}$  in 0.01 M PBS containing 0.015 M NaCl, pH 7.0) and dried in a refrigerator at 4 °C (referenced to as Hb/Au/GACS/GCE).

### 2.5. Treatment of real samples

The process of treatment included several steps. First, pickled radish was stirred adequately in a juicer, and then 12.5 mL of saturated borax solution and some of hot double-distilled water were added slowly to 5 g of the pretreated pickled radish. Afterwards, the mixture was shaken gently followed by heating at

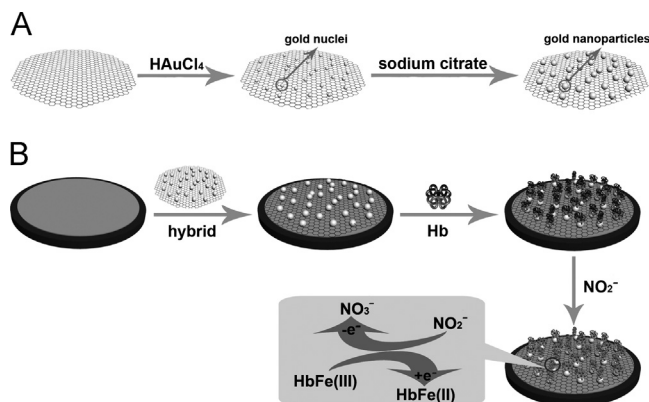


Fig. 1. Schematic illustration of preparations of (A) graphene-AuNP hybrids and (B) Hb-immobilized graphene-AuNP biosensor.

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