Applied Surface Science 355 (2015) 587–592

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Palladium nanoparticles deposited on graphene and its electrochemical performance for glucose sensing

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 20 July 2015
Accepted 21 July 2015
Available online 26 July 2015

Keywords:
Glucose biosensor
Glucose oxidase
Reduced graphene oxide
Palladium nanoparticles
Electrochemical

ABSTRACT

This paper reports on the fabrication and characterization of glucose oxidase (GOx) immobilized onto a glassy carbon electrode (GCE) modified with reduced graphene oxide/palladium nanocomposite (RGO–Pd). Characterization tools showed well dispersed uniform Pd nanoparticles on a partly reduced graphene oxide surface. Cyclic voltammetry demonstrated successful immobilization of GOx on RGO–Pd modified GCE (GCE–RGO–Pd) using covalent bonding of GOx with RGO–Pd (RGO–Pd–GOx). Therefore, it was used as an electrochemical biosensor of glucose. RGO–Pd–GOx exhibited good electrocatalysis toward glucose in different glucose concentrations (from 2 to 10 mM, which includes the blood glucose levels of both normal and diabetic persons) with O_2 saturated phosphate buffer solution (PBS) at pH 7.4. The system showed a linear increase in current at potential −0.085 V in the concentration range examined, with a correlation coefficient of 0.996. The sensitivity of the biosensor was 41.3 μA cm^{-2} mM^{-1}, suggesting that RGO–Pd–GOx-modified GCE could be a potential candidate as a glucose sensor.

1. Introduction

Diabetes mellitus is considered to be one of the most serious diseases affecting human health in developed countries, with complications including increased risk of heart disease, kidney failure, and blindness [1,2]; as such, the detection of glucose is important in the treatment and management of diabetes, which results in failed regulation of glucose levels. So far, several sensing methods have been proposed to detect glucose, including electrochemical [3] and optical [4] sensing. The electrochemical method has many advantages, such as its high sensitivity, good selectivity, fast detection and low cost. However, the major challenge in the fabrication of electrochemical glucose sensors is the immobilization of the model enzyme, glucose oxidase (Gox), on the electrode surface through effective electrical communication between GOx and the transducer, without compromising the mechanical stability and catalytic activity of the enzyme [3]. To overcome these issues, diverse methods and materials have been developed for the surface modification of electrodes [5,6].

Graphene, a one-atom thick carbon nanomaterial, represents a new two-dimensional (2D) material which has the unique mechanical and transport properties needed for a wide range of technologies [7]. In particular, graphene shows outstanding electron transport properties due to its 2D hexagonal crystal structure and the presence of charge carriers behaving like massless particles [8–10]. In addition, graphene is characterized by extremely high in-plane stiffness – Young modulus and superior (highest ever measured) strength [11]. Therefore, in recent years graphene has shown promising applications in bioelectronics and biosensors [12–14]. Shamsipur and Tabrizi [15] have used electrochemically reduced graphene oxide/sodium dodecyl sulphate composite to immobilize glucose oxidase and investigated it as an electrochemical glucose biosensor. The apparent heterogeneous electron transfer rate constant (k_s) of GOx at the electrode surface was estimated to be 4.1 s^{-1}. The resulting biosensor exhibited a good response to glucose with a linear range from 1 to 8 mM ($R2 = 0.9878$), good reproducibility and a detection limit of 40.8 μM. The dependence of the formal potential on the pH of the solution indicated that the direct electron transfer reaction of GOx was a two-proton

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http://dx.doi.org/10.1016/j.apsusc.2015.07.150
0169-4332/

coupled with a two-electron redox reaction process. The proposed biosensor was able to catalyze the reduction of dissolved oxygen, and glucose determination was achieved based on a decrease of peak currents due to the reduction of dissolved oxygen. Mani et al. [16] have immobilized glucose oxidase onto graphene–cobalt-phthalocyanine composite and used it for enzymatic determination of glucose. The amount of electroactive GOx (Γ) and electron transfer rate constant were calculated to be 3.77×10^{-10} mol cm^{-2} and 3.57 s^{-1}, respectively. The fabricated amperometric biosensor detected glucose in a wide linear concentration range from 10 μM to 14.8 mM with a high sensitivity of 5.09 μA mM^{-1} cm^{-2}. The sensor offered a very low detection limit (LOD) of 1.6 μM. F. Qu et al. [17] have designed a glucose biosensor based on a dual-path electron transfer mechanism. In this biosensor, ferrocenecarboxylic acid (FcCA) – the indirect mediator – transferred the electrons of the enzymatic reaction from the embedded redox active center via a redox process. Reduced graphene oxide (RGO) and silver nanoparticles were the conductive materials, providing a fast direct electron transfer path. In this system, β-cyclodextrin was of key importance for not only reducing RGO and Ag, but for providing a biocompatible microenvironment for the enzyme as well. Concurrently, carboxymethyl-β-cyclodextrin (CM-β-CD) formed an inclusion complex with FcCA and then covalently bound with the enzyme to immobilize FcCA and GOx. The rate constant (k_s) of the modified electrode was calculated to be 4.648 s^{-1}, indicating a fast electron transfer rate on the electrode. The glucose biosensor exhibited a wide linear response range from 0.105 to 11.805 mM with a low detection limit of 0.035 mM.

In this study we used a glassy carbon electrode, modified with reduced graphene oxide/palladium nanocomposite, with glucose oxidase immobilized onto the top of its surface, as an electrochemical glucose biosensor.

2. Materials and methods

2.1. Preparation of graphene oxide (GO)

Graphene oxide (GO) was synthesized using a modified Hummers method [18]. 1 g of graphite and 6 g of $KMnO_4$ powders were placed in a round-bottom flask. A mixture of 120 mL of H_2SO_4 and 15 mL of H_3PO_4 was then slowly added through reflux. The reaction mixture was heated to 50 °C and stirred with a magnetic stirrer for 24 h. Subsequently, the mixture was cooled to room temperature, afterwhich 1 mL of H_2O_2 (30%) was slowly added. The mixture was purified via sequential washing and centrifugation with water, HCl aqueous solution (1:3) and ethanol, to remove metal ions and attain a neutral pH value.

2.2. Functionalization of GO with Pd nanoparticles

75 mg of GO powder was dispersed in 150 mL of aqueous solution of ethylene glycol (1:2) and bath sonicated for 2 h to obtain a homogeneous suspension. 200 mg of $Pd(O_2CCH_3)_2$ powder was dissolved in an aqueous solution of ethylene glycol (1:2) and the resulting solution was added to the GO suspension, followed by sonication for 1 h. Afterwards, the mixture was vigorously stirred at 110 °C for 24 h under reflux. The final product, reduced graphene oxide functionalized with palladium nanoparticles (RGO–Pd), was repeatedly washed with water and ethanol, filtrated and dried with air at 60 °C.

2.3. Preparation of electrode

The glassy carbon electrodes (GCEs, d = 5 mm) were polished with 1.0 μm, 0.3 μm, and 0.05 μm alumina powders in turn. During these steps, deionized water was used to rinse the polished electrodes. After drying with air, the working electrodes were ready for use. About 30 μL of graphene oxide or reduced graphene oxide-palladium (RGO–Pd) nanocomposites (1 mg/mL) water solution was dropped onto the surface of the GCEs and dried with air for 12 h. The coated GCEs were then put into a 10 mL tube containing 500 μL glucose oxidase (GOx) solution (10 mg/mL), 10 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) to react at 4 °C for 24 h. In the control group, blank GCE was directly put into 500 μL of GOx solution (10 mg/mL) for 24 h. In the final step, the modified GCEs were rinsed with PBS to remove the unbounded GOx and dried at 4 °C for 12 h. The prepared GCEs were kept in a refrigerator before use.

2.4. Characterization

Transmission electron microscopy (TEM) (FEI Tecnai F30) was employed to examine the morphology of the graphene oxide and reduced form samples. FT-IR absorption spectra were acquired with the Nicolet 6700 FT-IR spectrometer for characterization of efficient oxidation and reduction of graphene. XRD was performed using the X'Pert Philips Diffractometer with a Cu anode ($K_{\alpha 1}$ = 1.54056 Å) to determine the crystal structure. Thermogravimetric analysis, used to verify the Pd loading and the functional group concentration, was performed on the SDT Q600 at an air flow of 100 mL min^{-1} and at a heating rate of 10 °C min^{-1}. Raman spectra, used to characterize the nanomaterials structure, were acquired on the inVia Raman Microscope (Renishaw) with an excitation wavelength of 785 nm.

2.5. Electrochemical sensing of glucose

Cyclic voltammetry measurements were performed on a VMP3 electrochemical analyzer (BioLogic Science Instruments), in phosphate buffered saline (0.05 M PBS pH 7.4) with a conventional three-electrode system: an Ag/AgCl electrode as the reference electrode, a Pt wire electrode as the auxiliary electrode and a modified glassy carbon electrode as the working electrode.

3. Results and discussion

Transmission electron microscopy (TEM) was used to characterize the morphology of the materials. Representative TEM images of GO and RGO–Pd nanocomposite are presented in Fig. 1(a)–(c). The TEM images indicate an excellent homogeneous distribution of palladium nanoparticles on the surface of the reduced graphene oxide flakes. The sample consists of a mixture of various shapes of palladium nanoparticles: spherical, triangular, cubic, hexagonal, rhomboidal and rod-like. A histogram presenting the diameter distribution of the Pd nanoparticles in RGO–Pd nanocomposite is presented in Fig. 1 (d). The diameter of the nanoparticles is in the range of 5–40 nm with a strong peak at 15 nm.

To examine the samples, Fourier transform infrared (FTIR) spectroscopy was employed. Fig. 2(a) depicts the IR spectra of GO and RGO–Pd nanocomposite. In the spectrum of GO, the following absorption modes were detected: 1026 cm^{-1} and 1099 cm^{-1} corresponding to the C—O stretching vibration mode in the alkoxy group, 1260 cm^{-1} attributed to the epoxy C—O stretching peak, 1452 cm^{-1} assigned to the C—OH carboxyl group, 1740 cm^{-1} arising from the C=O stretch mode in the carboxyl group, 2960 cm^{-1} originating from C—H, and 3426 cm^{-1} indicating the presence of O—H groups [19]. The spectrum confirms the successful oxidation of graphite. In the IR spectrum of RGO–Pd nanocomposite only two peaks from oxygen-containing functional groups can be noticed, suggesting the partial reduction of the GO during the deposition of Pd nanoparticles.