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Direct electrochemistry of glucose oxidase at electrochemically reduced graphene oxide-multiwalled carbon nanotubes hybrid material modified electrode for glucose biosensor

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ABSTRACT

Direct electrochemistry of glucose oxidase (GOx) at an electrochemically reduced graphene oxide–multiwalled carbon nanotubes hybrid (ERGO–MWCNT) modified glassy carbon electrode (GCE) has been reported. The π–π stacking interaction operating between the MWCNT and graphene oxide (GO) has been revealed by UV–Vis absorption spectroscopy. GOx was well immobilized onto the ERGO–MWCNT hybrid film, as a result direct electrochemistry of GOx has been achieved. Compared with pristine MWCNT, 2.1 fold higher peak current and very low peak to peak separation (ΔE_p) of 26 mV were observed at the hybrid film, demonstrating faster electron transfer between GOx and the modified electrode surface. Moreover, the modified film exhibited high electrocatalytic activity towards glucose via reductive detection of oxygen consumption and in the presence of mediator. The proposed biosensor exhibits low detection limit of 4.7 μM with wide linear range of 0.01–6.5 mM and acquires excellent storage and operational stabilities. The accurate glucose determination in human blood serum and good recoveries achieved in spiked urine samples revealed their great potential in the practical applications.

1. Introduction

Graphene, a monolayer of sp^2 bonded carbon atoms with versatile electronic, mechanical and thermal properties has been emerged as a promising material in diverse fields of research. Owing to its interesting physicochemical properties, it finds wide range of applications including electronics, super capacitors, batteries, fuel cells and biosensors (Geim and Novoselov, 2007; Pumera et al., 2010; Yoo et al., 2008). Although graphene can be prepared by several methods, most of them are not suitable for mass production and processing, which limit its applications (Chen et al., 2010). One viable method for the bulk production of graphene is chemical method, involving chemical oxidation of graphite to graphene oxide (GO) and subsequent reduction (Hummers and Offeman, 1958). GO, the oxygenated derivative of graphene is an amphiphilic molecule, in which hydrophilicity makes it highly dispersible in water, whereas hydrophobicity provides active sites for the interaction with other aromatic compounds (Dreyer et al., 2010).

On the other hand, carbon nanotube (CNT) viewed as a rolled graphene sheets also exhibits excellent mechanical, electrical and electrocatalytic properties (Wang, 2005). In the past decade, massive amount of works have been done on both multiwalled carbon nanotube (MWCNT) and single walled carbon nanotube (SWCNT) based electrochemical sensors and biosensors (Jacobs et al., 2010). Although, it can be easily dispersed in organic solvents, it tends to irreversible agglomeration in a short period of time because of Van der Waals interactions between the side walls. To overcome this shortcoming, several strategies have been developed in the past using surfactants, ionic liquids, polymer wrapping and chemical functionalization approaches (Kachoosangi et al., 2009; Kang and Taton, 2003). But still, a thirst to explore a better dispersant for MWCNT is everlastingly increasing among the researchers.

Being a versatile dispersant, GO has a unique ability to form stable aqueous dispersions, highly bio-compatible and possess good electrocatalytic properties (Ramesha and Sampath, 2007). In addition, its reductive product graphene is a unique material with potential applications in diverse fields and one of the hottest materials of interest. Zhang et al. (2010) demonstrated that it is possible to prepare a water dispersible GO-MWCNT hybrid material via non-covalent π–π stacking interactions. Dispersing MWCNT via non-covalent approaches cannot depress its intrinsic

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electrical, mechanical, and optical properties. The incorporation of GO with MWCNT render this hybrid material as a versatile platform for the electrocatalytic applications. Conversely, the insulating property of GO caused by the aliphatic sp^3 hybridized domain, limits its conductivity. Therefore for improving the conductance, it can be reduced to graphene either by chemical, thermal or electrochemical methods (Gao et al., 2010). Often chemical methods involve the use of highly toxic reductant hydrazine (Mani et al., 2012), whereas thermal methods require high temperatures. On the other hand, electrochemical approach involved neither toxic reducing agents nor high temperature, rather considered to be versatile, fast, efficient and green. Recent studies as well reveal that the electrochemical reduction of GO to graphene was appreciably improved after the incorporation of MWCNT (Qiu et al., 2010).

Acute monitoring of glucose is of significant importance and it has been successively achieved by using GOx based biosensors. Till now, numerous approaches have been developed for the GOx immobilization based on CNT modified electrodes. Some of the reported literature including CNT-gold colloid modified electrode (Yao and Shiu, 2008), gelatin-MWCNT (Periasamy et al., 2011), polymerized ionic liquid-wrapped CNT (Xiao et al., 2010), CNT-ionic liquid composite (Kachoosangi et al., 2009), nitrogen doped MWCNT (Deng et al., 2009), MWCNT–GOx (Gutierrez et al., 2012) and boron-doped CNT (Deng et al., 2008). Other than CNTs, several strategies were employed for the GOx immobilization based on graphene based modified electrodes. These approaches include polyvinyl pyrrolidone-protected graphene/polyethylenimine-functionalized ionic liquid (Shan et al., 2009), graphene-Au-nafion biocomposite (Zhou et al., 2010), nitrogen-doped graphene (Wang et al., 2010), GOx–graphene–chitosan (Kang et al., 2009) and chitosan–ferrocene/GO (Qiu et al., 2012).

In this report, for the first time we exploited ERGO–MWCNT hybrid material for the effective immobilization of GOx and studied its direct electrochemistry. Furthermore, the prepared modified electrode has been demonstrated as a versatile glucose biosensor. The accurate glucose determination achieved in human blood serum and spiked urine samples were proved the great ability of the sensor in the practical applications.

2. Experimental

2.1. Reagents and apparatus

GOx, type x–s from aspergillus Niger, graphite (powder, $< 20\ \mu m$), MWCNT (bundled $> 95\%$, O.D × I.D × length of 7–15 × 3–6 × 0.5–200 μm) were purchased from Sigma-Aldrich and used as received. 5 wt% nafion (Nf) was purchased from Aldrich and the required Nf concentrations were prepared in definite volume of ethanol. The mediator ferrocene mono carboxylic acid (FMCA) was purchased from Alfa Aesar. All the reagents were of analytical grade and used without purification. The supporting electrolyte used for the electrochemical studies was 0.1 M phosphate buffer solution (PBS), prepared using Na_2HPO_4 and NaH_2PO_4 and the pH was adjusted either using H_2SO_4 or NaOH. Prior to each experiment, all the solutions were deoxygenated with pre-purified N_2 gas for 15 min unless otherwise specified.

The electrochemical measurements were carried out using CHI 611A work station. Electrochemical studies were performed in a conventional three electrode cell using BAS GCE as a working electrode (area 0.07 cm^2), saturated Ag/AgCl as a reference electrode and Pt wire as a counter electrode. Amperometric measurements were performed by an analytical rotator AFMSRX (PINE instruments, USA) with a rotating disc electrode (RDE) having working area of 0.24 cm^2. UV–Vis absorption and Fourier transform infrared (FT-IR) spectroscopic measurements were carried out using Hitachi U-3300 spectrophotometer and Perkin Elmer spectrum RXI, respectively. EIM6ex ZAHNER (Kroanch, Germany) was used for electrochemical impedance spectroscopy (EIS) studies. Field emission scanning electron microscopy (FESEM) was performed using JEOL JSM-6500F.

2.2. Preparation of GO–MWCNT hybrid material

Graphite oxide was synthesized by modified Hummer's method (Hummers and Offeman, 1958) and it was exfoliated through ultrasonication for 2 h to get graphene oxide (GO). The as-obtained aqueous GO solution was subjected to centrifugation for 30 min at 4000 rpm to remove any unexfoliated graphite oxide. Thereafter, 5 mg of MWCNT was added into 10 mL of GO solution (0.5 mg mL^{-1}) and the mixture was subjected to ultrasonication for 2 h. An unstabilized MWCNT and excess of GO were removed by subjecting two consecutive centrifugation cycles (30 min each) at 8000 and 14,000 rpm, respectively, and the residue was assigned to be GO–MWCNT hybrid (Zhang et al., 2010). Thus obtained hybrid material was dried overnight, re-dispersed in water (0.5 mg mL^{-1}) and used for further studies. The dispersion is highly stable for more than 6 months. As a control, GO and pristine MWCNT dispersions (0.5 mg mL^{-1}) were prepared in water and DMF solvents, respectively.

2.3. Fabrication of ERGO-MWCNT /GOx/Nf modified GCE

GCE surface was polished with 0.05 μm alumina slurry using a Buehler polishing kit, then washed with water, ultrasonicated for 5 min and dried. 5 μL of GO–MWCNT dispersion was drop casted onto the pre-cleaned GCE and dried at room temperature. Thereafter, GO–MWCNT modified GCE was transferred to an electrochemical cell containing 0.1 M PBS (pH 5). The GO–MWCNT was electrochemically reduced by performing 3 consecutive cyclic voltammograms briefly explained in the Section 3.2. Then 4 μL (10 mg mL^{-1}) of GOx was spread gently onto the surface of ERGO–MWCNT modified GCE and dried at ambient temperature. The GOx modified surface was smoothly washed with water to remove loosely adsorbed enzyme. Finally 2 μL (0.5%) of Nf was drop casted and the fabricated ERGO–MWCNT/GOx/Nf film modified GCE was dried. For comparison, ERGO/GOx/Nf and MWCNT/GOx/Nf modified GCEs were prepared by adopting the similar procedures.

3. Results and discussion

3.1. Characterization of as synthesized ERGO–MWCNT

The FESEM image of the GO–MWCNT hybrid film at 100 nm resolution is shown in Fig. 1A. It can be seen that thin GO sheets were completely wrapped onto the tubular network of MWCNTs. The possible interaction is π–π stacking interaction between the hydrophobic region of GO and the sidewalls of MWCNT. This interaction avoids the restacking of MWCNT and keeps the GO–MWCNT dispersion stable for long time. Further, UV–Vis absorption spectroscopy has been used to confirm the π–π interactions involved between GO and MWCNT. Fig. 1B depicts the UV–Vis spectra of GO (a) and GO-MWCNT (b). The UV–Vis spectrum of GO exhibits two characteristic absorption peaks. A broad absorption peak at 229 nm assigned to π–π^* transition and a shoulder peak at 280 nm assigned to n–π^* transitions. Interestingly, the π–π^* transition of GO appeared at 229 nm was red shifted to higher wavelength of 248 nm (Bathochromic Shift) in the spectrum of GO-MWCNT. Hence, the interaction should be π–