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Multiwalled carbon nanotubes dispersed in carminic acid for the development of catalase based biosensor for selective amperometric determination of H_2O_2 and iodate

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ABSTRACT

We report the preparation of stable dispersion of multiwalled carbon nanotubes (MWCNTs) using carminic acid (CA) as a dispersing agent. The transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) results confirmed that MWCNT is well dispersed in CA aqueous solution and CA has been well adsorbed at MWCNT walls. Fourier transform infrared (FTIR) and UV–vis absorption spectra results also confirmed the adsorption of CA at MWCNT. To develop a highly selective amperometric biosensor for H_2O_2 and iodate, the model enzyme catalase (CAT) was immobilized at CACNT modified glassy carbon electrode surface. The immobilized CAT exhibits well defined quasi reversible redox peaks at a formal potential ($E^{\circ\prime}$) of −0.559 V in 0.05 M pH 7 phosphate buffer solution (PBS). The proposed CAT/CACNT biosensor exhibits excellent amperometric response towards H_2O_2 and iodate in the linear concentration range between 10 μM to 3.2 mM and 0.01–2.16 mM. The sensitivity values are 287.98 $\mu A\,mM^{-1}\,cm^{-2}$ and 0.253 $mA\,mM^{-1}\,cm^{-2}$, respectively. Moreover, the developed CAT biosensor exhibits high affinity for H_2O_2 and iodate with good selectivity.

1. Introduction

Hydrogen peroxide (H_2O_2) is an essential component of plant tissues, which regulates the plant metabolism, defense, acclimatory processes and gene expression (Slesak et al., 2007). Moreover, H_2O_2 has excellent oxidizing and antibacterial property and it has been widely employed in industries as an oxidizing agent (Yeh et al., 2003), antibacterial agent (Weston, 2000) and bleaching agent (Moore and Argyropoulos, 1999). Owing to the critical role played by H_2O_2 in biological systems as well as its wide applications in diverse fields, it has been extensively studied these days. On the other hand, iodine is the most vital micronutrient which is obligatory for the well-being of all individuals. In recent years, iodine deficiency is a worldwide reported serious health problem and it impersonates solemn threats to all stages of human life. Predominantly, in pregnant woman its deficiency leads to cretinism and in the case of older children and adolescents the temporary iodine insufficiency causes reduced physical and academic performance (Ketpichainarong et al., 2010). Nevertheless, one of the main factors accountable to iodine deficiency is the dietary inadequacy of iodine. To date the most suitable method to avoid iodine deficiency is fortification of salt with iodine (Ahad and Ganie, 2010).

It is exigent to achieve sensitive H_2O_2 and iodate determinations in routine analysis by the existing conventional approaches (Matsubara et al., 1985; Jackson and Hewitt, 1996; Kazuaki, 1997; Bichsel and Gunten, 1999; Zarei, 2009). Hence, there has been colossal demand for developing novel H_2O_2 and iodate quantification methods with simple and cost-effective measurement protocols. Conversely, by employing versatile electrochemical techniques in concert with the implementation of enzyme based biosensors highly sensitive H_2O_2 and iodate quantifications have been achieved these days (Salimi et al., 2007a). Catalase (CAT) is an active enzyme of oxidoreductase family which catalyzes the disproportionation process of H_2O_2 and it has been widely employed in H_2O_2 based biosensors (Prakash et al., 2009a). Multiwalled carbon nanotubes (MWCNTs) with their exceptional stability, good electrical conductivity and high mechanical strength (Srivastava and Wei, 2003) have been used as compelling immobilization matrices for CAT to accomplish sensitive H_2O_2 detection and for promoting the direct electron transfer of CAT (Prakash et al., 2009a). Earlier, we reported the direct electrochemistry of CAT at nafion (NF) wrapped MWCNTs in the presence of didodecyldimethylammonium bromide (DDAB) through electrostatic interactions between CAT, MWCNT-NF and DDAB (Prakash et al., 2009b).

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On the other hand, because of the hydrophobic nature and Van der Waals attractions between the nanotubes, it is always difficult to prepare stable aqueous dispersion of MWCNTs. This imperative shortcoming necessitates the need for exploring novel dispersing agents with versatile strategies (Kharisov et al., 2009). The most widely employed strategy is the attachment of functional groups at MWCNT walls through covalent and noncovalent approaches (Tasis et al., 2006). Even if stable MWCNT dispersion could be prepared by acid treatment, larger agglomerates and longer tubes with poorer graphene layers will break at a highest rate during acid treatment (Wang et al., 2003). To avoid this, in majority of the cases noncovalent approach has been preferred. The dispersing agents often used in noncovalent approach are surfactants (Madni et al., 2010), polymers (Liu et al., 2006), DNA (Li et al., 2009a) and biomolecules (Moulton et al., 2005). However, several parameters including surfactants/monomer concentrations and compatibility of the biomolecules have to be optimized. Especially, storage stability of the MWCNT dispersions prepared by the above stated methods are of great concern, when biomolecules are used as dispersing agents. Alternatively, by using simple ultrasonication treatments stable dispersion of MWCNT can be prepared by dispersing them in organic dyes through strong π-π stacking interactions. Besides, this kind of strategy is simple, economical, color variations of dye solutions could be visually observed and the MWCNT-dye adducts formed could be confirmed by several available spectroscopic and surface morphological studies (Zhang and Silva, 2010).

Carminic acid (7-D-glucopyranosyl-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid or CA), is the active color ingredient of natural dye cochineal produced by *Dactylopius coccus*, a cochineal insect (Gaweda et al., 2008). As shown in Fig. S1, structure of CA possesses an anthraquinone moiety linked to glucose sugar unit. CA is non-toxic, possess antitumor and antioxidant property (Li et al., 2009b), and used as a coloring agent in various food stuffs (Lancaster and Lawrence, 1996). Moreover, CA shows good biocompatibility for various proteins and it undergoes strong interactions with biomolecules (Sun et al., 2006). Besides, CA is a compound of great electrochemical importance. Several reports about the electrochemical (Grygar et al., 2003; Carbo et al., 2003), impedance behavior (Rashwan, 2005) and spectroscopic analysis (Rasimas and Blanchard, 1995; Canamares et al., 2006) of CA are available in literature.

However, to the best of our knowledge no reports are available about the preparation of stable aqueous dispersion of MWCNT using CA as a dispersing agent. Moreover, CA functionalized MWCNT have not been used as an immobilization platform for CAT. In this work, we adapted a noncovalent approach with simple ultrasonication treatment for preparing more stable aqueous dispersion of MWCNT using CA as a dispersing agent. The π-π stacking interactions between CA and MWCNTs provide good stability to the CACNT dispersion. We utilized the prepared CACNTs as a novel matrix for CAT immobilization and to explore its direct electrochemistry. CAT immobilized at CACNT modified electrode shows good affinity and high selectivity towards H_2O_2 and iodate with appreciable sensitivity.

2. Experimental

2.1. Reagents

Carminic acid was purchased from Sigma–Aldrich. MWCNT with O.D. 10–15 nm, I.D. 2–6 nm and length 0.1–10 μm was obtained from Aldrich. CAT from bovine liver (4540 units mg^{-1}) was purchased from Sigma. H_2O_2 (30%) and potassium iodate were obtained from Wako Pure Chemical Industries, Ltd. The supporting electrolyte used for all experiments is 0.05 M pH 7 phosphate buffer solution (PBS). All aqueous solutions were prepared using doubly distilled water. Prior to each experiment, the solutions were deoxygenated with pre-purified N_2 gas for 10 min and N_2 tube was kept above the solutions to maintain an inert atmosphere.

2.2. Apparatus

Cyclic voltammetry experiments were carried out using CHI 1205a work station. GCE with an electrode surface area of 0.079 cm^2 was used as working electrode. Pt wire with 0.5 mm diameter was used as counter electrode and standard Ag/AgCl was used as a reference electrode. Amperometry studies were performed using CHI 750 potentiostat with analytical rotator AFMSRX (PINE Instruments, USA). UV–vis absorption and fourier transform infrared (FTIR) spectroscopy measurements were carried out using Hitachi U-3300 spectrophotometer and Perkin Elmer spectrum RXI. EIM6ex ZAHNER (Kroanch, Germany) was used for electrochemical impedance spectroscopy (EIS) studies. Surface morphology studies were performed using JEM 2007 model transmission electron microscope (TEM), Hitachi S-3000 H scanning electron microscope (SEM) and Being nano-instruments CSPM 4000, atomic force microscope (AFM).

2.3. Preparation of CACNTs

About 10 mg of MWCNT was added into 10 ml of 5 mM CA aqueous solution and the whole mixture was ultrasonicated for 1 h, until a homogeneous slight reddish-black dispersion was obtained. CACNT dispersion was then filtered and the solid material was subjected to several washings with water and over night dried at 50 °C in an air oven. Thus obtained CACNTs were dissolved in aliquots of double distilled water and sonicated well to obtain a final concentration of 1 mg ml^{-1}. For comparison, without any CA addition 1 mg of as-received MWCNT was dispersed in 1 ml of doubly distilled water and sonicated well. Hereafter, the term CNT used elsewhere in this paper will represent the non-functionalized MWCNT-water dispersion.

3. Results and discussion

3.1. Investigation of long term storage stability and surface morphological characterizations

In order to evaluate the long term storage stability, prepared CACNT and CNT dispersions were stored in two separate small micro centrifuge tubes, kept at room temperature and were examined regularly. The photographs taken at various intervals are shown in Fig. 1(a–m). No notable precipitation of nanotubes bundles was observed in CACNT dispersion even after four months (see Fig. 1(m)). This result validates the good storage stability of CACNT dispersion and it could be ascribed to the strong π-π interactions between MWCNT walls and CA. In contrast, CNT dispersion displayed poor storage stability, where CNT bundles precipitated completely after 1 month (see Fig. 1(j)). The poor storage stability of CNT dispersion can be ascribed to the hydrophobic nature and strong Van der Waals attractions between the individual nanotubes.

The surface morphology of prepared CNT and CACNT dispersions were comparatively studied using TEM. As shown in Fig. 1A, TEM image of CNT dispersion shows few well defined CNTs with an O.D. of 10 nm. The black spots found at CNT walls might be the metal catalyst impurities. However, in the TEM image of CNT took at higher magnifications more agglomerates were found (figure not shown). Whereas, as shown in Fig. 1(B–D), TEM images of CACNT dispersion took at 20 nm and 5 nm magnifications displays the well dispersed