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Hollow graphitic nanocapsules as efficient electrode materials for sensitive Hydrogen peroxide detection

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ABSTRACT

Carbon nanomaterials are typically used in electrochemical biosensing applications for their unique properties. We report a hollow graphitic nanocapsule (HGN) utilized as an efficient electrode material for sensitive hydrogen peroxide detection. Methylene blue (MB) molecules could be efficiently adsorbed on the HGN surfaces, and this adsorption capability remained very stable under different pH regimes. HGNs were used as three-dimensional matrices for coimmobilization of MB electron mediators and horseradish peroxidase (HRP) to build an HGN–HRP–MB reagentless amperometric sensing platform to detect hydrogen peroxide. This simple HGN–HRP–MB complex demonstrated very sensitive and selective hydrogen peroxide detection capability, as well as high reproducibility and stability. The HGNs could also be utilized as matrices for immobilization of other enzymes, proteins or small molecules and for different biomedical applications.

1. Introduction

Carbon nanomaterials have attracted wide attention based on their unique physical and chemical properties. Different kinds of carbon-based nanomaterials have been developed, such as fullerene, carbon nanotubes (CNTs), graphene, carbon dots, and carbon cages (Smalley, 1992; Tans et al., 1998; Chen et al., 2008; Novoselov et al., 2004; Chen et al., 2010; Sun et al., 2006; Sheng and Wang, 2008; Yang et al., 2010). Among these materials, graphitic nanocapsules, as a type of carbon cage, have recently been explored for such applications as lithium ion batteries (Wang et al., 2005), catalysts (Wu et al., 2007; Wu et al., 2008; Schaefer et al., 2010), supercapacitors (Bushueva et al., 2008; Xie et al., 2012), immunoassays (Cui et al., 2008; Ho et al., 2009), and drug delivery (Uo et al., 2005). Unlike other amorphous carbon nanocapsules, graphitic nanocapsules have properties similar to those of graphene and carbon nanotubes, such as unique structures, very high conductivity, and good mechanical and biocompatible properties (Hwang, 2010). Their high surface area, good conductivity and three-dimensional structure could make graphitic nanocapsules a good electrode material for electrochemical biosensing. Graphitic nanocapsules also have high chemical stability and excellent dispersion characteristics which are important both for optimizing synergistic nanoparticle-support interactions and maximizing the mass activity of expensive precious enzymes. However, many applications and properties unique to graphitic nanocapsules remain to be explored. For example, increasing interest has been shown in controllable synthesis of graphitic nanocapsules and constructing bio-hybrid nanocomposites and structures for broader biomedical applications.

In particular, hydrogen peroxide is a universal molecule and has significant functions as a signaling molecule in the regulation of a variety of biological processes, including aging and carcinogenesis (Veal et al., 2007; Giorgio et al., 2007; López-Lázaro, 2007). As such, the accurate determination of hydrogen peroxide is essential in the biological, environmental and clinical fields. Accordingly, many methods have been developed for the highly sensitive detection of hydrogen peroxide. The electrochemical method is a widely used approach by its simplicity and high sensitivity, different strategies and electrode systems have been explored, such as HRP-peptide on gold electrode (Zhao et al., 2013), HRP–Au–chitosan–clay (Zhao et al., 2008), HRP–gold nanowire (Xu et al., 2010), HRP–attapulgite on glassy carbon (GC) (Wu et al., 2011), HRP–CNT–chitosan–sol–gel (Kang et al., 2009), HRP–CNT–methylene blue (Xu et al., 2003; Zhang et al., 2010), Pt on glassy carbon (O'Neil et al., 2004), Pt–MnO–graphene (Xiao et al., 2012), TiO_2–cytochrome c (Luo et al., 2009), and PtPd–Fe_3O_4 (Sun et al., 2012). While these methods have all demonstrated their utility, it is worthwhile exploring new electrode materials and developing a simpler and more effective approach to fabricate reagentless amperometric biosensors for hydrogen peroxide. One such candidate is the graphitic nanocapsule.

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Herein, we developed a method of synthesizing hollow graphitic nanocapsules (HGN) and using them as an electrode material for hydrogen peroxide detection. These HGNs were used as three-dimensional matrices to effectively immobilize enzymes, proteins and small molecules. Specifically, a reagentless amperometric biosensor was created by coimmobilization of methylene blue (MB) electron mediators and horseradish peroxidase (HRP) enzyme on the HGN-coated electrode. The positively charged HRP and MB molecules were anchored on the HGN surface through electrostatic adsorption. As a consequence of the extra strong π–π interaction, HGNs exhibited a high loading capacity for the MB electrochemical mediator-molecules. The constructed HGN–HRP–MB platform ably facilitated electron shuttling between the active center of HRP enzyme and the surface of the electrode. Thus, by using HGN as the electrode matrix, together with a simple MB and HRP assembly strategy, the sensing platform demonstrated the ability to detect hydrogen peroxide with high sensitivity, selectivity, reproducibility and stability. Indeed, hydrogen peroxide molecules could be detected less than 1 μM.

2. Experimental

2.1. Chemicals

Methylene blue and horseradish peroxidase (300 U/mg) were obtained from Shanghai Reagent Company (Shanghai, China). All other chemicals of analytical reagent grade or higher were obtained from Changsha Chemical Reagents Company (Changsha, China) and used as received without further purification. All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore). The buffer used in this work was 10 mM phosphate buffer solution (PBS) and 0.15 M NaCl (pH 7.4).

2.2. Preparation of HGN

Hollow graphitic nanocapsules were obtained from a core-shell magnetic graphitic nanomaterial (MG). MG was synthesized with a chemical vapor deposition (CVD) system as reported previously (Chen et al., 2012; Song et al., 2013). Briefly, we first impregnated fumed silica (1.00 g, Aladdin) with $Co(NO_3)_2 \cdot 6H_2O$ (2.06 g) in methanol. Then the methanol was removed, the mixture dried, and the powder ground. Typically, 0.50 g of the powder was used for methane CVD growth in a tube furnace at 800 °C for 5 min. After growth, the sample was etched with 10% HF in H_2O (80%) and ethanol (10%) to dissolve the silica. We collected the MG solid product through centrifugation and washed thoroughly. To obtain the HGN, a solution of sulfuric and nitric acid was utilized to polish the as-received MG for 4 h and solubilize it in water. The excess MGs were removed by an external magnet. The HGNs were collected through centrifugation and washed thoroughly with ultrapure water.

2.3. Adsorption measurement of methylene blue on HGN

Adsorption measurements were performed with simple mixing of the MB and HGN solutions. Typically, the MB solution at a concentration of 2 mg/L was used for the preparation of MB–HGN nanocomposites through a premixing procedure with different amounts of HGNs. The mixture was shaken for 30 min at room temperature and then centrifugated at 7000 rpm for 5 min. The resulting supernatant was collected, and UV–vis spectra were recorded using a Shimadzu UV-2550 spectrophotometer (Shimadzu International Trading Co., Ltd., Shanghai, China).

2.4. Preparation of HGN–HRP–MB-modified electrode

Prior to modification, the bare glassy carbon electrode was thoroughly polished with emery paper and alumina slurry in the order of 1.0, 0.5, and 0.03 μm, followed by ultrasonication in water. Next, the electrode was immersed in a freshly prepared piranha solution (30% H_2O_2 and 98% H_2SO_4, 1/3, v/v) for 40 min. After this, the electrode was rinsed with ultrapure water and electrochemically pretreated with cyclic potential scanning to obtain a clean glassy carbon electrode. Then, 5 μL, 1.75 mg/mL HGN was cast on the electrode surface and air-dried at room temperature. Following this step, the electrode was soaked in 10 mg/mL HRP solution and incubated overnight. Meanwhile, a homogeneous solution (I) composed of 1 mg/mL HRP and 0.33 mg/mL MB was prepared in PBS (pH 7.4). The HGN–HRP–MB electrode was obtained by dipping the HRP-coated HGN glassy carbon electrode into solution I (PBS, pH 7.4) for 12 h at 4 °C, followed by careful rinsing with ultrapure water. Finally, 5 μL of 1% Nafion ethanolic solution was cast on the electrode surface and air-dried at room temperature to ready the instrument for electrochemical measurements.

2.5. Characterization and electrochemical measurements

Raman spectroscopy was performed on a Horiba Jobin Yvon LabRAM-010 Raman microscope with 632 nm He–Ne laser excitation. The hydrodynamic diameters of the HGNs under investigation were measured using a Zetasizer Nano ZS90 Dynamic Light Scattering (DLS) system equipped with a red (633 nm) laser and an Avalanche photodiode detector (APD) (quantum efficiency > 50% at 633 nm) (Malvern Instruments Ltd., Worcestershire, England). Zeta potential measurements were performed in water. The measurements were carried out at room temperature on the ZetaSizer Nano ZS90 equipped with MPT-2 Autotitrator and 4 mW He − Ne laser (λ_0 = 633 nm) using the Laser Doppler Electrophoresis technique. Electrochemical measurements were carried out with a CHI 760 electrochemical analyzer (CH Instrument Company, Shanghai, China). A conventional three-electrode cell was used. A bare or modified glassy carbon electrode (GCE) was used as a working electrode, and a platinum disk was used as an auxiliary electrode, with a saturated calomel electrode (SCE) as reference.

3. Results and discussion

3.1. Characterization of hollow graphitic nanocapsules

The prepared HGNs were analyzed by scanning electron microscopy (SEM), as shown in the images pictured in Fig. 1A and B. These HGNs showed uniform size distribution. Transmission electron microscopy (TEM), which was utilized for the structural characterization of HGNs, showed a hollow structure with some wrinkles on the surface (Fig. 1C). In the high-resolution TEM image of the HGN (Fig. 1D), the graphitic shell structure was clearly observed, and the intralayer distance of the shell was around 0.34 nm, which is consistent with that of graphite. The digital camera image shows the graphitic nanocapsule in water solution before and after the hollow structure was prepared (Fig. 1E). After the acid treatment, the HGNs form a black suspension which could remain stable for months.

The HGNs were further characterized with selected area electron diffraction (SAED) and Raman spectroscopy, and both techniques indicated the graphitic structure of the nanocapsules. As demonstrated in Fig. 2A, the SAED diffraction patterns can be assigned to the (002), (100), (004) and (112) facets of the hexagonal crystalline graphite, respectively. The graphitic shell was also proved through Raman