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Synthesis of highly dispersed titanium dioxide nanoclusters on reduced graphene oxide for increased glucose sensing

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ABSTRACT

Highly dispersed titanium dioxide nanocluster (TDN) was synthesized on reduced graphene oxide (RGO) in a toluene–water system under microwave irradiation. The prepared RGO–TDN hybrids were used to modify glassy carbon electrode for loading glucose oxidase. The fabricated glucose biosensor exhibits excellent performance for glucose sensing including low work potential (−0.7 V), high sensitivity (35.8 μA mM^{-1} cm^{-2}), low detection limit (4.8 μM), wide linear range from 0.032 to 1.67 mM, small Michaelis–Menten constant (K_m) (0.81 mM), and short response time (10 s).

1. Introduction

Due to its superior properties such as large specific surface area, high uniformity and excellent biocompatibility, nanoarchitectured TiO_2 has attracted considerable interest and has been applied in a variety of fields including photocatalysis [1–3], fuel cells [4–6] and biosensors [7]. Recently, it has been proven that the modification of electrode with different TiO_2 nanostructures can increase the catalytic performance of enzyme for promising biosensor applications. For example, TiO_2 nanocrystal [8,9], TiO_2 nanotube [10] and TiO_2 nanofibers [11] were used as the substrate of glucose biosensor. The physicochemical properties of TiO_2 nanostructures largely depend upon their sizes, crystalline forms and aggregation states [12]. As an immobilization substrate for biomolecules, the size of TiO_2 particles was an important factor [9]. Small TiO_2 particles are favorable for loading enzymes on their surface. Great efforts have been made to synthesize TiO_2 with controllable morphology and small size. For the past few years, template-based nanoengineering methods have been extensively studied, in which many different materials such as porous alumina [13,14], polymer gel [15], surfactant [16–18], activated carbon [19], carbon fiber [20] and carbon nanotube [21,22] have been used as templates for preparing TiO_2 with different kinds of nanostructures.

Graphene, a dense honeycomb crystal structure packed by a monolayer of sp^2 hybridized carbon atoms [23], has been received increasing attentions during recent years because of its unique physicochemical properties including high surface area [24], excellent electric conductivity [25], strong mechanical strength, ease of functionalization and mass production [26]. Compared with carbon nanotube (CNTs), graphene has shown lower noise and higher sensitivity in the applications as electrochemical sensor and biosensor [27]. Graphene oxide (GO) or reduced graphene oxide (RGO) has been used as a novel template for preparing and stabilizing of Ag [28], Au

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[29], Pt [30] and Pd nanoparticles [31] with many potential applications. There were also some researches about the synthesis of TiO_2 nanoparticles on RGO [32–39], but those researches mainly focused on the photocatalysis or lithium storage of RGO–TiO_2 hybrids. As far as glucose sensing concerned, TiO_2 nanostructure with smaller size on RGO would be more interesting, and there was still no report about the synthesis of TiO_2 nanocluster (TDN) on RGO, especially by microwave.

Herein, highly dispersed TDN was synthesized on RGO for the first time under microwave irradiation. In order to explore the advantages of RGO–TDN hybrids in the adsorption of enzyme and keeping its bioactivity, glucose oxidase (GOD) was chosen as a model enzyme and an amperometric GOD biosensor was fabricated by loading GOD on glassy carbon electrode (GCE) modified with RGO–TDN hybrids. The electrochemical catalytic activity of the fabricated electrode in response to glucose was investigated. Due to the special property of RGO–TDN hybrids, it is expected that an excellent glucose biosensor based on RGO–TDN hybrids will be fabricated.

2. Experimental

2.1. Preparation of GO

Natural graphite was used for the preparation of GO. Graphite oxide was prepared from natural graphite powder by Hummers and Hoffman's method [40] with some modifications. In brief, 1 g of graphite powder and 30 mL of sulfuric acid were added into a reaction vessel in a dry ice bath, and stirred gently for 6 h. Then 3 g of potassium permanganate was added slowly with violent stirring. The reaction was allowed to proceed at below 20 °C for 30 min and at 35 °C for 30 min. Then 30 mL of deionized water was added into the reaction vessel slowly, and the reaction was kept at ~95 °C for 35 min. At last 140 mL of deionized water and 10 mL of 30% hydrogen peroxide were added into the reaction vessel for finishing the reaction. The resulting graphite oxide was filtered, and washed with 5% hydrochloric acid and deionized water to remove the free SO_4^{2-}. The graphite oxide was suspended in the deionized water, and exfoliated through ultrasonication for 3 h. The yellow–brown upper solution was GO, which was to be used as the resource of preparing RGO–TDN hybrids.

2.2. Synthesis of highly dispersed TDN on RGO

0.05 mL of *tert*-butylamine was added into 5 mL of GO aqueous colloidal solution (~0.3 mg ml^{-1}) and the mixtures were transferred to a quartz tube. Subsequently, 0.075 g of titanium (IV) isopropoxide and 1.0 mL of oleic acid (OA) were dissolved in 5 mL of toluene in air, and the mixed solution was transferred to the same quartz tube. In this way, the two-phase system containing water and toluene was formed. Then the quartz tube was sealed and maintained at 150 °C for 3 h with magnetic stirring under microwave irradiation. When the reaction was finished, the color of aqueous colloidal solution changed from yellow brown to black. The reaction was still kept with stirring for 3 h at 150 °C to ensure RGO combined with Ti center of TDN. The as-prepared solution was purified with a great deal of acetone and centrifuged at 10,000 rpm for 10 min to get RGO–TDN hybrids. Then the obtained RGO–TDN hybrids were washed with 100 mL of tetrahydrofuran to get rid of residual OA thoroughly.

2.3. GCE modified with RGO–TDN hybrids and GOD

GCE (3 mm diameter) was polished successively with 1.0, 0.3, and 0.05 μm α-alumina powders. After successive ultrasonication in ethanol and deionized water, the electrode was rinsed with deionized water and dried at room temperature. The modified GCE was prepared by a simple casting method. 20 mg mL^{-1} RGO–TDN suspension was prepared by dispersing 10 mg of RGO–TDN hybrids in 0.5 mL of deionized water with ultrasonic agitation for about 10 min. To accomplish the preparation of GCE modified with RGO–TDN hybrids and GOD (GCE/RGO–TDN/GOD/CS), 5 μL of RGO–TDN suspension (20 mg mL^{-1}) was dropped on the surface of GCE and dried in air. 5 μL of GOD (10 mg mL^{-1}) was then coated on GCE modified with RGO–TDN hybrids (GCE/RGO–TDN) and dried at 4 °C to form GCE modified with RGO–TDN hybrids and GOD (GCE/RGO–TDN/GOD). Finally, 10 μL of chitosan solution (5 mg mL^{-1}) was dropped on GCE/RGO–TDN/GOD and dried at room temperature. The modified electrode was immerged into 0.1 M phosphate buffered saline (PBS) (pH 7.0) to remove the loosely adsorbed GOD and was stored at 4 °C in a refrigerator under dry conditions for further use. For comparison, GCE modified with dissociating TiO_2 in toluene (DTD) and GOD (GCE/DTD/GOD/CS) was prepared by the similar procedure.

2.4. Characterization and measurements

Transmission electron microscopy (TEM) and high resolution TEM (HRTEM) images were obtained using a JEOL JEM-2100F electron microscope operated at 200 kV. The sample was prepared by dropping RGO–TDN hybrids on micro-grids supported on a 200 mesh copper grid and allowing deionized water to dry for observation. The X-ray powder diffraction (XRD) characterization was performed on a D/max-B diffractometer. Raman spectra of samples were measured by using a Renishaw INVIA Reflex Raman micro-spectrometer with 514 nm diode laser excitation on a 1800 lines/mm grating at room temperature.

The electrochemical experiments were performed with a CHI660d electrochemical workstation (CHI, Austin, TX). All measurements were conducted at room temperature (25 °C) with a conventional three-electrode cell, which included a Ag/AgCl (saturated by 3 M KCl solution) as reference electrode, a platinum wire as counter electrode, and a bare or modified GCE (3 mm in diameter) as working electrode. Cyclic voltammograms (CVs) were carried out at a scan rate of 100 mV s^{-1} in a quiescent PBS (pH 7.0), which had been purged with high-purity nitrogen or oxygen for at least 20 min prior to experiments. Amperometric experiments were carried out in O_2-saturated PBS (pH 7.0) with successive adding glucose solution at the work potential of −0.7 V.

3. Results and discussion

RGO–TDN hybrids were synthesized by an easy and one-pot method. The main feature of this method is the efficient