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Preparation of poly(diallyldimethylammonium chloride)-functionalized graphene and its applications for H_2O_2 and glucose sensing

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ABSTRACT

In this study, poly(diallyldimethylammonium chloride) (PDDA) was selected as an electron acceptor for functionalizing graphene. The resultant PDDA functionalized graphene (PDDA-G) was characterized using TEM, UV–vis absorption spectrum, Raman spectrum and electrochemical method. The PDDA-G modified electrode displayed remarkable electrocatalytic activity toward H_2O_2 reduction and can be used as a nonenzymatic biosensor for H_2O_2 detection. Then negatively charged glucose oxidase (GOD) was immobilized onto the positively charged PDDA-G matrix driven by electrostatic interaction. This novel GOD/PDDA-G bionanocomposite can be used as a biosensor for the detection of glucose with a linear range from 0.02 to 1.8 mM and a detection limit of 8 μM. In this report, the biosensors are easy to prepare, have good stability, and will have potential applications in H_2O_2 and glucose sensing.

1. Introduction

Carbon-based materials, such as diamond, graphite, fullerenes, graphene, and carbon nanotubes, have been widely used in both analytical and industrial electrochemistry due to their wide potential window, relatively inert electrochemistry and electrocatalytic activity for a variety of redox reactions. Among these, carbon nanotubes (CNTs) and graphene have been shown to be the hottest topics of materials research. CNTs as nanoscale building blocks have been employed for sensors and electronic devices [1,2]. But the only drawback of CNTs as nanofiller is their high production cost [3]. Therefore, the mass production of CNTs-based functional composite materials is very difficult. As Nicholas A. Kotov wrote in his review in *Nature* [4] "when carbon fibers just won't do, but nanotubes are too expensive, where can a cost-conscious materials scientist go to find a practical conductive composite? The answer could lie with graphene sheets". Graphene is considered two dimensional carbon nanofiller with a one-atom-thick bonded carbon atoms that are densely packed in a honeycomb crystallattice. It is regarded as the thinnest material in the universe with tremendous application potential [5,6]. Graphene is predicted to have remarkable properties, such as high thermal conductivity, superior mechanical properties and excellent electronic transport properties [7–11]. However, just as other newly discovered allotropes of carbon, material availability and processability will be the rate-limiting steps in the evaluation of extensive applications of graphene. For graphene, its availability is encumbered by having to surmount the high cohesive van der Waals energy (5.9 kJ mol^{-1} carbon) [12] adhering graphitic sheets to one another. So the surface modification of graphene is an essential step for obtaining a molecular level dispersion of individual graphene. The functional groups attached to graphene can be small molecules [13–16] or polymerchains [17–19]. The chemical functionalization of graphene is a particularly attractive target because it can improve the solubility and processability [20]. Considerable work has been carried out on the amination [21], esterification [22], isocyanate modification [23] and polymer wrapping [17–19] as routes for the functionalization. Poly(diallyldimethylammonium chloride) (PDDA), a linear positively charged polyelectrolyte, has been found to be attractive for functionalizing nanomaterials [24–26]. Accordingly, functionalizing graphene with PDDA should be an effective method to increase the solubility for the extending application in biosensing.

The accurate and rapid determination of H_2O_2 is of practical importance due to its application in food, pharmaceutical, clinical, industrial, and environmental analysis. To date, a great amount of enzymatic biosensors [27–29] were developed to detect H_2O_2. However, enzymatic sensors cannot provide the admitted advantage of a complete long-term stability due to the intrinsic nature of

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enzymes. Therefore, it is necessary to develop a simple nonenzymatic strategy for sensing H_2O_2 with high sensitivity. It has been proved that metal nanoparticles show good electrocatalytic activity toward the H_2O_2 reduction [30,31], which provides another strategy for the nonenzymatic determination of H_2O_2. Whereas, the high cost of metal nanoparticles such as Pt, Au, *etc.*, together with their limited reserve in nature, has shown to be the "bottleneck" for large-scale commercialization of this kind of biosensors. Here we prepared PDDA-functionalized graphene sheets (PDDA-G) by the reduction of graphene oxide with hydrazine as reducing agent in the presence of PDDA, in which PDDA prevented the aggregation of graphene sheets through electrostatic repulsion and improved the dispersibility of graphene in water. Meaningfully, the PDDA-G composite showed higher electrocatalytic activity toward H_2O_2 reduction than pure graphene. The nonenzymatic biosensor based on the PDDA-G nanocomposite showed wide linear range and low detection limit for the detection of H_2O_2.

Glucose is a key metabolite for living organisms, especially in the case of patients suffering from diabetes. Since the first enzyme electrode was reported in 1962, more efforts have been contributed to glucose biosensors based on glucose oxidase (GOD). For instance, many researchers used carbon nanotubes (CNTs) as electron transfer relay between GOD and electrode substrate to construct glucose biosensors [32,33]. Investigations of glucose biosensors based on graphene materials are booming recently [34,35]. In the present study, we found that GOD can be firmly immobilized onto the surface of PDDA-G nanocomposite *via* the electrostatic interaction without any other cross-linking reagents. A pair of well-defined redox peaks of the immobilized GOD was obtained, indicating the PDDA-G composite was facile to promote the direct electron transfer of GOD. The combination of the unique property of graphene with the excellent biocompatibility of PDDA provided a promising platform for the fabrication of mediator-free enzymatic biosensor. The method presented here was easy and feasible, which provided a model for the immobilization of other enzymes/proteins.

2. Experimental

2.1. Reagents

Graphite powders (325 meshes, 99.9995%) were purchased from Alfa Aesar Company. Glucose oxidase (from Aspergillus niger, 50 ku) was obtained from Sigma. Other reagents such as $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, KCl, P_2O_5, $K_2S_2O_8$, H_2SO_4, H_2O_2, $KMnO_4$, $H_4N_2{\cdot}H_2O$, $C_6H_{12}O_6{\cdot}H_2O$, Poly(diallyldimethylammonium chloride) (MW = 200,000–350,000) were obtained from Tianjin guangfu chemical reagents company. All reagents were of analytical grade and without any further purification. Double distilled water was used in the whole experimental process.

2.2. Apparatus and measurements

Transmission electron microscopy (TEM) micrographs were obtained using a JEOL2000 transmission electron microscopy operating at 200 kV. Raman spectra were recorded on an RM3000-514 Laser Raman Spectrometer (Renishaw Instruments, England) with 514 nm laser excitation. The UV–vis absorption spectra were obtained using a Shimadzu UV-2550 spectrophotometer.

All electrochemical measurements were performed on a CHI 760 electrochemical workstation (Shanghai, China) with a conventional three-electrodes system composed of a platinum auxiliary, a Ag/AgCl (saturated KCl) reference, and a glassy carbon working electrode. A 0.1 M phosphate buffer solution (PBS) consisted of Na_2HPO_4 and NaH_2PO_4 was employed as supporting electrolyte.

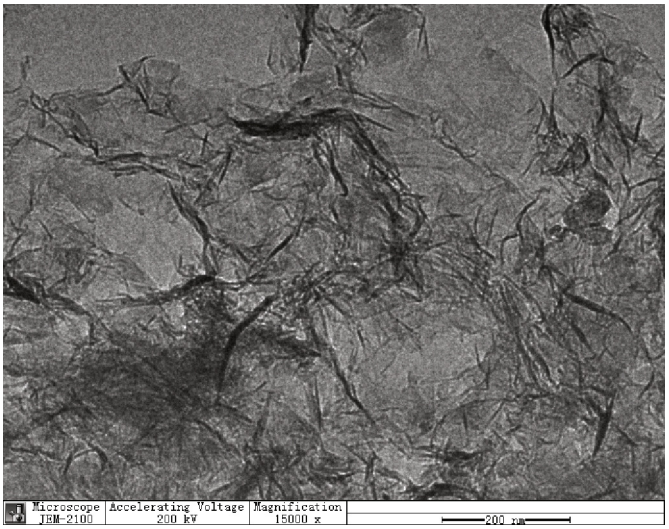

Fig. 1. TEM of PDDA functionalized graphene.

2.3. Synthesis of PDDA-functionalized graphene

Graphene oxide (GO) was prepared from graphite powder by the modified Hummers method [36] and then dispersed in water to yield a yellow-brown dispersion by ultrasonication for 3 h, followed by centrifugation at 4000 rpm for 10 min to remove any unexfoliated GO. Subsequently, the homogeneous GO dispersion (1 mg mL^{-1}, 50 mL) was mixed with 1 mL PDDA (20%) solution and stirred for 30 min. The resulting mixture was further treated with 1.5 mL hydrazine hydrate and allowed to react for 3 h at 100 °C in Teflon autoclave. Finally, the black PDDA-functionalized graphene (PDDA-G) was collected by centrifugation at 10,000 rpm for 10 min and further washed with water.

2.4. Preparation of PDDA-G/GC electrode and GOD/PDDA-G/GC electrode

Glassy carbon electrode (GCE, 3 mm, diameter) was polished to a minor with 1.0 and 0.3 μm alumina slurry sequentially on a polishing cloth and then washed with water and ethanol, respectively. The cleaned GCE was dried with high-purify nitrogen steam for the next modification. The PDDA-G/GCE was prepared as follows: 5 μL PDDA-G (1 mg mL^{-1}) was spread onto the GCE surface and dried naturally. The GOD/PDDA-G/GCE was prepared as follows: GOD was adsorbed onto the PDDA-G film by immersing the PDDA-G/GCE into 0.1 M phosphate buffer solution (pH 7.0) containing 10 mg mL^{-1} GOD for 5 h (4 °C). Then the enzyme electrode (GOD/PDDA-G/GCE) was rinsed with distilled water to remove the loosely bound enzyme molecules and was used for electrochemical measurements. The modified electrode was stored at 4 °C in a refrigerator if not be used. For comparison, the GOD/PDDA/GCE and GOD/GCE were prepared with a similar method.

3. Results and discussion

3.1. Characterization of PDDA-functionalized graphene (PDDA-G)

A TEM image of the PDDA-G, as shown in Fig. 1, distinctly illustrated that PDDA-G consists of the randomly aggregated thin sheets with wrinkles. These wrinkles may be the key point leading to a gain in elastic energy for the quasi-two dimension crystallite to avoid dislocations caused by thermal fluctuations and keep a metastable state [37]. The graphene oxides and PDDA-G were characterized with UV–vis spectroscopy. The UV–vis spectrum