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Graphene Nanosheets/Poly(3,4-ethylenedioxythiophene) Nanotubes Composite Materials for Electrochemical Biosensing Applications

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ABSTRACT

In this study, we developed the novel composite materials containing reduced graphene oxide (rGO) nanosheets and poly(3,4-ethylenedioxythiophene) nanotubes (PEDOT NTs) for electrochemical biosensing applications. Transmission electron microscopy, scanning electron microscopy and atomic force microscopy suggested that the rGO nanosheets cover the substrate uniformly, and the PEDOT NTs act as a conducting bridge to connect the rGO sheets. By combining the two materials, it's expected to enhance the conductivity of the film and improve the surface coverage. We applied the rGO/PEDOT NTs composite for electrochemical detection of hydrazine and hydrogen peroxide; noticeable improvements in electrochemical activity and reactivity were observed compared to those of the pristine rGO and PEDOT NTs electrodes. This may be attributed to the better surface coverage of the rGO/PEDOT NTs modified electrode with superior conductivity. Furthermore, interference tests indicate that the rGO/PEDOT NTs composite film exhibits high selectivity toward the analyte. The rGO/PEDOT NTs composite thus provides a potential platform for biosensing applications.

1. Introduction

As a two-dimensional sheet of sp2-bonded carbon atoms, graphene has received much attention in recent years on its structure, preparation, functionalization and application [1–4]. Graphene has good electron transport property [5] and high surface area [6], so it becomes a good candidate for biosensing application, such as detection of DNA, metal ion [7–10], protein and pathogen [3,11–15]. Besides, an ideal graphene sheet provides a good substrate for anchoring π-conjugated molecules through π-π stacking interaction [10]. Thus, desirable materials can be introduced to endow the graphene sheets to develop a novel composite for various applications.

Recently, the conducting polymers, including polypyrrole, polythiophene, polyaniline and their derivatives [16–20], have owned their potential for biomaterial application. Poly(3,4-ethylenedioxythiophene) (PEDOT) has been considered as one of the most promising conducting polymers because of the conductivity and stability provided by its ordered and well-defined chemical structure [21]. Especially, the nanotube appearance of PEDOT possesses higher one-dimensional conductivity and superior stability. However, nanotubes are not perfect candidates for the coating materials of modified electrodes, because most nanotube-based materials would suffer from the weak adhesion and poor coverage when coated on the substrates, thus resulting in the detachment from the substrates under stimulation [22,23]. In this study, we incorporated rGO nanosheets, which exhibit superior two-dimensional conductivity and uniform coverage on the substrate with PEDOT nanotubes (PEDOT NTs) to improve the coverage of the modified electrode.

Hydrazine (N_2H_4) and its derivatives are widely used in industry and agriculture, such as a reactant in fuel cells, rocket propellant, antioxidant, herbicide and pesticide [24]. Although hydrazine is a general-purpose chemical, but it is recognized as an environmental pollutant because of its high toxicity and irritation. In excess of exposure to hydrazine, it would harm the central nervous system

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and raise the probability for cancer [25]. Thus, fast, accurate and sensitive detection of hydrazine becomes an important issue. Various traditional analytical methods have been reported for detection of hydrazine, such as gas chromatography [26], chemiluminescence [27] and spectrophotometry [28], but these methods are usually expensive and time-consuming; complicated sample pretreatments are normally required in these analytical approaches. Compared to these reported methods, electrochemical methods possess many advantages such as cost-effectiveness, high sensitivity, and simple operation. Therefore, the electrochemical methods become a preferable choice to detect hydrazine.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species, widely used in food, pharmaceutical, industrial, and environmental fields and also an important product of oxidized-enzyme. Under proper concentration of H_2O_2, it is useful to kill the bacteria and viruses entering the body. In excess, it could damage the biological macromolecules such as DNA, carbohydrates or proteins and cause the cancer, even affect the fetus of pregnant women. Thus, the relationship between H_2O_2 concentrations and human physiology has attracted intensive study [29,30]. The accurate determination of H_2O_2 becomes more important in the clinical, environmental and industrial fields, so the study gradually focused on the fabrication of reliable H_2O_2 biosensors. Because of the high selectivity and sensitivity, electrochemical methods have been used extensively to detect the H_2O_2. Besides, biosensors were usually fabricated using enzymes or proteins such as horseradish peroxidase, cytochrome c, and hemoglobin [31–34]. However, enzyme-based biosensors lack long-term stability [35] and there have been extensive efforts to develop enzyme-free sensors [36–38].

Here, we prepared the novel composite material of rGO nanosheets and PEDOT NTs which comprises their unique conducting properties and makes up the disadvantages of individual material. The rGO/PEDOT NTs films exhibit much better electrochemical and electrocatalytic activities toward the analyte. Furthermore, interference tests of the composite films also reveal high selectivity toward the analyte. Thus, the rGO/PEDOT NTs composite materials can be applied for novel types of highly sensitive and selective enzyme-free biosensors.

2. Experimental

2.1. Chemicals

Graphite powder (Bay Carbon Inc. (USA), SP-1), poly(3,4-ethylenedioxythiophene) nanotubes (PEDOT NTs), hydrazine (Hyd), glucose (Glu), nitrite (Nit), serotonin (Ser), ascorbic acid (AA), dopamine (DA), uric acid (UA), l-cysteine (l-cys), hydrogen peroxide (H_2O_2) and methanol (MeOH) were purchased from Sigma–Aldrich. All other chemicals were of analytical grade and used without further purification. Aqueous solutions were prepared using doubly distilled water. 0.1 M phosphate buffer solution (PBS, pH 7), which was prepared from Na_2HPO_4 and NaH_2PO_4 solutions, was chosen as the supporting electrolyte for the electrochemical studies. All solutions were deoxygenated by purging with pre-purified N_2 gas.

2.2. Apparatus and measurement

Cyclic voltammetry (CV) was performed using a CHI 440 analytical system (CH Instruments) and its compatible software. A conventional three-electrode cell assembly, consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode, was used for the electrochemical measurements. The working electrode was either an unmodified glassy carbon electrode (GCE) or a GCE modified with materials. A rotating disk electrode (RDE) based on a GCE (working area: 0.2472 cm^2, Part #AFE7R9GCGC, PINE Instrument Company, Pennsylvania, USA) was modified with materials and dried at ambient temperature. All CVs were done against the Ag/AgCl reference electrode and the reported potentials were expressed in V with respect to the standard hydrogen electrode (SHE). The surface morphologies of the composites were investigated using scanning electron microscopy (SEM, Hitachi S-4700). The PEDOT NTs and rGO/PEDOT NTs solution were dropped on a holey carbon-coated copper grid (Lacey Carbon Type-A 300 mesh copper grid; TED Pella) and then dried in air at 70 °C prior to characterization using TEM (JEM 2100F). An alpha 300 Raman spectrometer (WITec Instruments, Germany) was used to analyze the compositions of the composite films with a fixed wavelength of 514.5 nm. The XPS spectra were recorded using a PHI 5000 VersaProbe (ULVAC-PHI, Chigasaki, Japan) system with He(I) ($h\nu = 21.2$ eV) as the energy source.

2.3. Preparation of rGO/PEDOT NTs modified electrode

The preparation of graphene oxide (GO) from graphite powder was following the modified version of Hummers' method [39]. Briefly, graphite powder (2 g), $NaNO_3$ (1 g), and H_2SO_4 (46 mL) were mixed in an ice bath and then $KMnO_4$ (6 g) was added slowly. Once mixed, the solution was transferred to a water bath and stirred at 35 °C for approximately 1 h, forming a thick paste. Water (80 mL) was added and then the solution was stirred for 1 h at 90 °C. Finally, more water (200 mL) was added, followed by the slow addition of H_2O_2 (30%, 6 mL). The warm solution was filtered and washed sequentially with 10% HCl (3 × 200 mL) and water (200 mL). The filter cake was dispersed in water through mechanical agitation and then stirred overnight. The dispersion was left to settle and the supernatant (clear yellow dispersion) subjected to dialysis for 1 month, resulting in a stock solution having a GO concentration of approximately 0.17 mg mL^{-1}. The stable dispersion was filtered through an alumina membrane and left to dry for several days. The GO paper was then carefully peeled from the filter and stored under ambient conditions [40]. To produce hybrid suspensions of rGO and PEDOT NTs, dry powders of GO and PEDOT NTs were dispersed directly in anhydrous hydrazine and left to stir for 24 h. Hydrazine solution contains the GO and PEDOT nanotubes powders for reducing the GO and soon formed a uniform dark gray suspension with no visible precipitation. After mixing the materials with hydrazine solution, the dark gray suspension was centrifuged to separate out the residual PEDOT NTs bundles and aggregated rGO. After centrifugation, the uniformity of the rGO/PEDOT NTs dispersion was ensured through heating at 60 °C with repeated ultrasonic agitation for approximately 30 min. Typically, a mixture of GO (1 mg mL^{-1}) and PEDOT NTs (3 mg mL^{-1}) in hydrazine was employed to prepare a modified electrode.

Bare GCEs were carefully polished by BAS polishing kit with aluminum oxide (Al_2O_3) powder and then rinsed with deionized water. The GCEs were drop-coated with rGO/PEDOT NTs dispersion and dried in an oven at 50 °C for 30 min. Then the obtained modified electrodes were washed carefully in deionized water to remove residual materials and solvent.

3. Results and discussion

3.1. Characterization of rGO/PEDOT NTs composite materials

The signals from resonance Raman spectra reflect the degrees of stacking and functionality of the PEDOT NTs, rGO and rGO/PEDOT NTs composites. In Fig. 1, PEDOT NTs show two strong bands at 1428 and 1506 cm^{-1}, which are attributed to the symmetric stretching mode and asymmetric stretching vibration of the –C=C–