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Nitrogen and sulfur dual-doped graphene for glucose biosensor application

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ABSTRACT

This work demonstrated the potential application of the N, S dual-doped graphene (NS-G) as a novel glucose oxidase (GOD) platform for glucose sensing. The NS-G was prepared by a simple two-step solvothermal method using urea as the N precursor and benzyl disulfide as the S precursor. The morphology and structure of as-prepared NS-G were characterized, and its electrochemical properties were examined. In comparison with the single N-doped graphene (N-G) based sensor, the dual-doped NS-G modified sensor showed significantly improved electrochemical sensing performances for glucose detection due to the synergistic effect of the coupling interactions between N and S heteroatoms that were responsible for the superior electrocatalytic properties. This study suggested that dual-doped graphene with two heteroatoms could be an effective way to greatly improve the electrochemical sensing performances of graphene-based GOD glucose biosensor.

1. Introduction

Glucose biosensors have received extensive attention over decades in academia and industries owing to their great importance in biology, food industry, environmental protection and clinical analysis [1–3]. Glucose oxidase (GOD) is a most common enzyme for the construction of enzyme glucose biosensors because of its low cost, bioactivity, and high sensitivity towards glucose detection [4,5]. It has been suggested that the major challenge in the development of high-performance GOD-based glucose biosensors is to achieve the direct electron transfer (DET) process between the active sites of GOD and the surface of electrode because the electroactive center of GOD, flavin adenine dinucleotide (FAD), is deeply buried inside the GOD shells, rendering them inaccessible for DET with bare electrodes [6–8]. In order to overcome this problem, a number of materials, including metal or metal oxide nanoparticles [9], conducting polymers [10], carbon nanomaterials, such as carbon nanotubes [11], carbon nanofibers [12] and graphene [13], and their composites [14,15] have been used to modify the electrode to immobilize GOD for improving the DET between GOD and electrode.

Graphene, a single layer of sp^2-bonded carbon atoms packed into a benzene-ring structure, has been demonstrated as an ideal electrochemical platform for the construction of GOD-based glucose biosensors owing to its large specific surface area, high electric conductivity, good biocompatibility, thermal and chemical stability, and low-cost production [13,16]. The graphene-based GOD biosensors have been prepared by a number of methods and used for electrochemical detection of glucose, exhibiting a high sensitivity and selectivity compared to most traditional GOD biosensors [13–15]. On the other hand, chemical doping of graphene with heteroatoms (N, P, B, S, Se, I, etc.) can effectively modulate its electrical properties and enhance its electrocatalytic performances [17–19]. It is suggested that the electrochemically active sites introduced by doped heteroatoms are favorable for the adsorption and activation of analytes, anchoring of functional moieties or molecules, and accelerating the charge transfer between electrode and analytes/electrolyte, all of which would be advantages to the enhanced electrochemical sensing performances [20]. Wang et al. [21] reported a glucose sensor based on N-doped graphene (N-G) modified GOD electrode, which exhibited a much stronger glucose response than undoped counterpart.

Most recently, some experimental studies have revealed that the dual-doping of N with other elements, such as S, B or P further increases the electrochemical properties due to the synergistic effect arising from the coupling interactions between two heteroatoms [22]. It has been reported that dual-doping graphene provides a more powerful electrocatalytic activity for oxygen reduction reaction (ORR) compared to that of single N-doped

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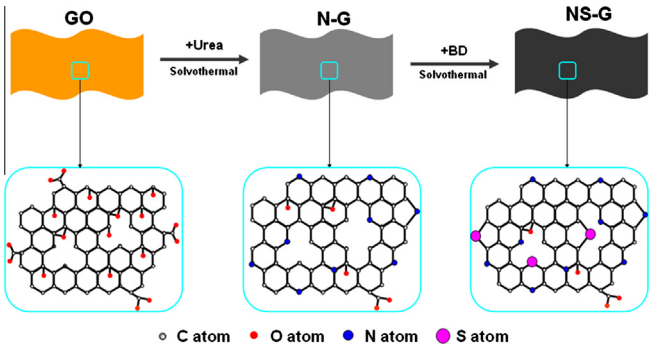

Fig. 1. Schematic diagram of the synthesis procedure of NS-G.

graphene (N-G) [23–26]. The performance of electrochemical sensors can also be notably improved by the use of dual-doped graphene materials. For instance, Yang et al. [27] prepared B, N dual-doped graphene modified sensor that outperformed the single-doped (B or N alone) graphene modified sensor for the highly sensitive detection of H_2O_2. Guo et al. [28] fabricated N, S dual-doped graphene modified sensor to simultaneously detect Pb^{2+} and Cd^{2+}. In comparison with single-doped (N or S alone) graphene, N, S dual-doped graphene could significantly improve sensitivity towards Pb^{2+} and Cd^{2+}. On the basis of the advantages of dual heteroatoms doping and encouraging reports, it is anticipated that the dual-doped graphene may well modify the electrode for improving the DET of GOD on the surface of electrode, which can be used for electrochemical detection of glucose. However, to our knowledge, no attention has been paid to employ dual-doped graphene as the GOD immobilization matrix for glucose detection.

In this study, we used a simple two-step solvothermal method (Fig. 1) to synthesize N and S dual-doped graphene (NS-G) that was tested as a novel GOD platform for the construction of GOD-based glucose biosensor. The morphology, crystal and chemical structure of as-prepared NS-G were characterized, and its electrochemical properties were examined in detail. Compared to the biosensor based on single-doped N-G, the NS-G modified biosensor was found to exhibit a better glucose sensing performances in terms of detection sensitivity, limit of detection, and linear range due to the synergistic effect of the coupling interactions between N and S heteroatoms to boost its electrocatalytic activity.

2. Experimental

2.1. Materials

Graphite powders were purchased by Qingdao Dongkai Graphite Co., Ltd. Glucose oxidase (GOD, Type X-S: from Aspergillus niger, 150,000 units/g) were purchased from Sigma–Aldrich. D-(+)-glucose and chitosan (85% deacetylation) were purchased from Shanghai Chemical Reagent Co. All other chemicals were purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China), and used without further purification. Phosphate buffer solution (PBS) was prepared freshly from Na_2HPO_4 and KH_2PO_4 reagents, and its pH value was adjusted with H_3PO_4 or NaOH.

2.2. Synthesis of NS-G

GO was prepared by chemical oxidation and exfoliation of natural graphite following the modified Hummer's method [29]. NS-G was synthesized by a simple two-step solvothermal method using urea as the N source and benzyl disulfide (BD) as the S source, as illustrated in Fig. 1. For the synthesis of N-G, 200 mg of GO was dispersed in 500 mL of deionized water under ultrasonication for 1 h. Then 2 g of urea was added into the GO suspension under ultrasonication for another 1 h. The above suspension was sealed in a Teflon-lined autoclave and treated at 170 °C for 8 h. After the autoclave was cooled down, the solid products were collected by centrifugation, washed repeatedly with distilled water and dried at 60 °C for 24 h. The NS-G was then prepared through the same procedure as N-G except that GO and urea were replaced by 50 mg of N-G and 0.5 g of BD (dispersed in 200 mL ethanol), respectively, and the solvothermal conditions were changed to 190 °C for 10 h.

2.3. Characterizations

The morphologies and structures of the samples were characterized by transmission electron microscopy (TEM, JEM-2100), high-resolution transmission electron microscopy (HRTEM, JEM-2100), X-ray diffraction (XRD, Bruker D8 Avance), X-ray photoelectron spectroscopy (XPS, PHI 5000C ESCA) and Raman spectroscope (Alpha 300R, WITEC). The nitrogen adsorption/desorption isotherms were recorded at the liquid nitrogen temperature (77 K) using a ASAP 2020M apparatus.

2.4. Electrochemical experiments

All the electrochemical measurements were conducted on a PGSTAT-302N electrochemical workstation using a conventional three-electrode system at room temperature. A bared or modified glassy carbon electrode (GCE, diameter 3 mm) was used as working electrode. A Ag/AgCl electrode and a Pt wire were used as reference and counter electrodes, respectively. Phosphate buffer solution deaerated with high-purity nitrogen or oxygen was employed as the running electrolyte. The modified GCE was prepared by a simple casting method. Prior to use, the GCE was highly polished to a mirror-like surface with alumina paste, followed by ultrasonically washing with distilled water, and allowed to dry in a stream of nitrogen. Then 5 mg of electrocatalyst (NS-G or N-G) was added to 2 mL of chitosan solution (0.5 wt.%, 2.0% acetic acid) to form a homogeneous dispersion under ultrasonication for 1.0 h. Next, 5 μL of the above suspension was cast onto the surface of the pretreated GCE and dried in air to form NS-G/GCE. After that, the modified NS-G/GCE was immersed in 0.1 M PBS (pH = 7.4) containing GOD (4 mg/mL) at 4 °C for 24 h to immobilize GOD on the electrode surface and obtain the GOD/NS-G/GCE. Finally, the modified electrode was rinsed thoroughly with water to wash away the loosely adsorbed GOD and air-dried at room temperature. All the as-prepared electrodes were stored at 4 °C in a refrigerator under dry conditions when not in use.

3. Results and discussion

3.1. Morphologies and structures of NS-G

Fig. 1 illustrates the formation mechanism of NS-G obtained by a two-step solvothermal synthesis. In brief, N-G is firstly prepared by solvothermal reaction of GO and urea (N source), where the interactions between oxygen-containing groups on GO and urea enable the formation of C—N bond [30]. Following the similar solvothermal conditions, in the second step, the residue oxygen-containing groups on N-G can react with BD (S source) to form additional C—S bond [31]. Consequently, the N, S dual-doped NS-G containing both C—N and C—S bonds is obtained. In the following, the morphologies and structures of as-prepared NS-G were fully characterized by TEM/HRTEM, XRD, Raman, and XPS techniques.

Fig. 2 shows the TEM images of the morphologies of as-prepared GO, N-G and NS-G samples. It is clear that the morphologies of the