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Preparation of graphene/nile blue nanocomposite: Application for oxygen reduction reaction and biosensing

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ABSTRACT

Nile blue/graphene (NB-GNs) nanocomposite was synthesized for the first time via a green and effective one-step electrochemical method, allowing to reduce graphene oxide (GO) and NB on the glassy carbon electrode (GCE) simultaneously and construct GCE-GNs-NB_{poly} composite. The composite was characterized by scanning electron microscopy (SEM), UV–Vis spectroscopy, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The electrochemical results obtained in the absence of any redox probe, where NB was active, allowed to trace step-by-step addition of the NB-GNs nanocomposite onto the GCE electrode surface, supporting formation of the GCE-GNs-NB_{poly} composite. The electrocatalytic activity of the as-prepared GCE-GNs-NB_{poly} towards O_2 reduction was studied in neutral medium. The results revealed excellent electrocatalytic performance for two-electron reduction of oxygen, suggesting its potential application as metal-free electrocatalysts for O_2 reduction reaction. Application of the GCE-GNs-NB_{poly} in electrochemical biosensing was demonstrated by immobilization of glucose oxidase (GOx) on the surface of GCE-GNs-NB_{poly}, and then, using it for sensing of glucose. The biosensor exhibited a linear response, from 0.2 to 2.0 mM glucose, with a low detection limit, 2.1 μM, and high sensitivity, 67.0 $\mu A\,mM^{-1}\,cm^{-2}$, obtained by cyclic voltammetry method. The proposed biosensor was successfully tested for determination of glucose in blood serum samples.

1. Introduction

Platinum-based catalysts have been widely investigated for oxygen reduction reaction (ORR), however, they suffer from disadvantages of prohibitively high cost and poor stability, which are becoming major obstacles for the commercialization in fuel cells [1–4]. Therefore, the search for non-precious-metal as well as metal-free catalysts has become one of the most active and competitive events in the field of fuel cells researches [5–8].

In the past two decades, many different carbon based materials, such as single walled and multiwalled carbon nanotubes, have been used as electrocatalyst supports in fuel cells [7–9]. However, graphene nanosheets (GNs), as a novel generation of carbon-based nanosheets with sp^2 hybrid carbon network, have been developed for various potential applications due to their exceptional electronic, optical, high charge mobility and large surface area properties [10–15].

The GNs have been employed as catalytic supports in fuel cells due to their unique advantages, such as the large surface area, novel electron transfer property, and thus, they have been incorporated into many functional materials to form nanocomposites for various applications in many aspects of electrochemical technology [16,17].

In particular, GNs based nanocomposites are of scientific and industrial interests due to the synergistic contribution of two or more functional components [18,19]. Up to now, various materials have been incorporated into GNs layers, including metal nanoparticles [20] and conducting polymers (CPs) [18,21]. Among them, modification of the GNs electrode by CPs has provided higher specific surface area, more active sites and higher stability than those modified with grafted monolayers. The CPs offer great advantages as fuel cell catalyst supports, such as good conductivity and mechanical properties, simplicity and rapidity of preparation by chemical and electrochemical methods, and good adhesion to the electrode base that make theme suitable supports for low temperature fuel cell catalysts [22,23,24]. In the past two decades, ORR on anthraquinone (AQ) and its derivatives as one of the interesting CPs, has been widely studied [25,26,27]. The results have shown that the surface-confined AQs are good non-precious metal electrocatalysts for ORR with high current efficiency.

However, there is an increasing interest to extend such studies to other materials, which may be suitable as host for the catalyst

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particles to develop new electrocatalysts. Nile blue (NB) is a phenoxazine dye with highly promising properties as a redox catalyst. NB supported on GCE [28] or carbon nanotubes as CPs [29,30] has been examined as a redox mediator for sensing and biosensing purposes. It seems highly promising to insert the NB molecules inside the GNs and combine NB with GNs to make a high performance catalyst for electrochemical purposes like fuel cells and biosensor.

In the present study, we report for the first time, a new one-step electrochemical approach to synthesize high quality NB-GNs nanocomposite onto the GCE by using GO and NB as the starting materials. The as-prepared composites were characterized using SEM and Uv–Vis techniques. An average value of 1.26×10^{-9} mol cm^{-2} was obtained for NB surface concentration based on anodic peak surface area of the CV recorded for confined redox reaction of NB and supported by the value (1.20×10^{-9} mol cm^{-2}) obtained by using EIS. The electrocatalytic activity of GCE-GNs-NB nanocomposite electrode was completely examined for ORR in neutral medium by using combination of methods including CV, where excellent activity was observed.

To further demonstrate the electrocatalytic efficiency and applicability of the modified surface, the glucose oxidase (GOx) was used as a model, immobilized on the GCE-GNs-NB$_{poly}$ and successfully tested for recognition of glucose in the presence oxygen.

2. Experimental

2.1. Material and reagents

Graphite powder, sodium nitrate, Glucose oxidase (GOx), (from Aspergillus Niger 20,000 units/g, EC 1.1.3.4), ($NaNO_3$), potassium permanganate ($KMnO_4$), sulfuric acid (H_2SO_4 98%) solution, hydrogen peroxide solution (H_2O_2 30%), NB and other chemicals were of analytical grade obtained from commercial sources (Sigma-Aldrich® or Merck®), and used without further purification. All solutions were prepared with double-distilled water. The testing solutions were saturated with high purity N_2 or O_2, depend on the experimental conditions. Stoke solutions of glucose (10 mM) were prepared in 0.1 M PBS, pH 7.4, and stored overnight at 4 °C before use.

2.2. Synthesis of nanocomposites and modifications of the GCE electrode surface

Graphene oxide (GO) was synthesized by the modified Hummers' method (see Supporting Information, Section 1) [31]. The GCE (0.0314 cm^2) was polished with alumina powder (0.3 down to 0.05 μm) on a polish cloth (Microcloth, PSA, 40-7212, Buehler®), and then, washed ultrasonically in ethanol and water, respectively, for a few minutes to prepare clean GCE. A set of clean GCE was prepared.

A 20 mL mixture including 2.0 mg mL^{-1} GO and 1.0 mg mL^{-1} of NB monomer was prepared in aqueous solution, stirred, and sonicated in an ultrasonic bath (Bandlin, HF 35 kHz) for 2 h to prepare *NB-GO composite suspension solution for deposition* (NB-GO-cssd). Also two other solutions were prepared in the same way, but without NB or GO, and named GO-cssd and NB-cssd solutions, respectively.

The working electrodes were prepared using clean GCE as follows:

(i) A 20 μL of NB-GO-cssd solution was dropped onto the clean GCE and allowed to be dried under air to attach GO-NB onto the GCE surface by means of *physical adsorption*, forming GCE-GO-NB.

(ii) The electrochemical cell (a usual three-electrode cell, Section 2.4) was filled with 0.1 M KNO_3 solution. The GCE-GO-NB was placed into the cell, its potential was cycled between 0.000 and −1.3 00 V vs. Ag/AgCl with a sweep rate of 20 mV s^{-1} at room temperature for ~7 cycles to reduce GO to GNs [18], and absorb the GNs and NBs onto the GCE *simultaneously* by *electrochemical* polymerization of NB [30]. The constructed electrode is named GCE-GNs-NB$_{poly}$ electrode.

(iii) Also, 20 μL the GO-cssd solution was dropped onto the clean GCE surface and allowed to dry and attach GO onto the GCE surface by means of *physical adsorption* to form GCE-GO electrode.

(iv) One set of the GCE-GO electrodes was treated as (ii) to reduce GO to GNs *electrochemically* and form GCE-GNs.

(v) Finally, 20 μL of the NB-cssd solution was dropped onto the clean GCE surface and allowed to dry and attach NB to GCE by means of *physical adsorption* to form GCE-NB electrode.

(vi) One set of the GCE-NB electrodes was *electrochemically* treated as (ii) to polymerize NB molecule and form GCE-NB$_{poly}$.

So, we have four sets of electrodes for investigation as follows: (i) GCE-GNs-NB$_{poly}$ (*electrochemically* treated GCE-GO-NB), (ii) GCE-GNs (*electrochemically* treated GCE-GO), (iii) GCE-NB$_{poly}$ (electrochemically treated GCE-NB, the NB is expected to be polymerized on GCE), and (iv) the clean bare GCE. All the electrodes were washed with distilled water and used for measurements.

For the fabrication of GCE-GNs-NB$_{poly}$-GOx, five microliters of GOx solution (5 mg mL^{-1}) was coated on the GCE-GNs-NB$_{poly}$ and dried at 4 °C in air.

2.3. Physicochemical characterization

Scanning electron microscopic (SEM) images were acquired using a scanning electron microscope (SEM, Hitachi S4160, Cold Field Emission, Japan). XRD analysis of the samples was performed with a Bruker D8 Advance powder diffractometer using Ni filtered Cu-Kα radiation. The UV–Vis spectrum was recorded on Mutispec-1501 Shimadzu Hyper UV–Vis spectrophotometer.

2.4. Electrochemical characterization

All the electrochemical studies are performed at 25 ± 2 °C using a three-electrode assembly glass cell (6.1415.150, Metrohm, Switzerland) including, a Ag/AgCl (3 M KCl) electrode as the reference, a large area Pt plate as the counter electrode (70 times larger than that of the working electrode), and the modified GCE disk as the working electrode. All the potentials are measured and reported vs. Ag/AgCl (3 M KCl). The CV and EIS measurements are carried out on Autolab Potentiostat/Galvanostat101 instrument equipped with a frequency response analyzer, and controlled by Nova 1.8 Software (Eco Chemie, Utrecht, the Netherlands). The EIS data were approximated using ZView2.3f® software, considering the fit criteria and using appropriate electrical equivalent circuit built in this software, from which electron transfer kinetics as charge transfer resistance (R_{ct}), double layer capacitance (C_{dl}) and solution resistance (R_s) were extracted. Details of the electrochemical experimental setup are presented in our previous reports [32,33].

Step-by-step *modifications* of the surface was traced (i) in a no reaction potential region where the surface was not electrochemically active, and thus, an external redox probe as Ferri-ferrocyanide was applied, and (ii) in the potential regions where adsorbed NB was electrochemically active (confined redox reaction).

Electrocatalytic *activity* of the GCE modified electrode with the prepared catalyst toward the ORR was studied both under stationary or rotation of the electrode where catalyst was constructed on a glassy carbon or a rotating disk electrode (GCE-RDE) using different rotation speed and at a potential scan rate of 10 mV s^{-1}.