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Synthesis of Graphene Oxide Nanosheet: A Novel Glucose Sensor Based on Nickel–Graphene Oxide Composite Film[1]

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Abstract—The graphene oxide (GO) nanosheets were produced by chemical conversion of graphite, and were characterized by transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FT-IR). An electrochemical sensor based on Ni/graphene (GR) composite film was developed by incorporating Ni^{2+} into the graphene oxide film modified glassy carbon electrode (Ni/GO/GCE) through the electrostatic interactions with negatively charged graphene oxide. The Ni^{2+}/graphene modified glassy carbon electrode (Ni/GR/GCE) was prepared by cyclic voltammetric scanning of Ni/GO/GCE in the potential range from –1.5 to 0.2 V at 50 mV s^{-1} for 5 cycles. The electrochemical activity of Ni/GR/GCE was illustrated in 0.10 M NaOH using cyclic voltammetry. The Ni/GR/GCE exhibits the characteristic of improved reversibility and enhanced current responses of the Ni(III)/Ni(II) couple. The introduction of conductive graphene not only greatly facilitates the electron transfer of Ni^{2+}, but also dramatically improves the long-term stability of the sensor by providing the electrostatic interactions. Ni/GR/GCE also shows good electrocatalytic activity toward the oxidation of glucose. The Ni/GR/GCE gives a good linear range over 10 to 2700 μM with a detection limit of 5 μM towards the determination of glucose by amperometry. This sensor keeps over 85% activity towards 0.1 mM glucose after being stored in air for a month, respectively. Furthermore, the modified sensor was successfully applied to the sensitive determination of glucose in blood samples.

Keywords: graphene oxide, glucose, nickel ions, electrocatalysis, modified electrode

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1. INTRODUCTION

Among these carbon nanomaterials, graphene [1], which is a two-dimensional monolayer of carbon atoms parked into a dense hexagonal network structure, has received considerable attention from both the experimental and theoretical scientific communities in the last several years [2]. Because of its unique electric, thermal and mechanical properties [3], graphene has provided strong potentials to synthesis of nanocomposite [4]. Graphene (GR) is a one-atom-thick planar sheet of sp^2-bonded carbon atoms that are densely packed in a honey-comb crystal lattice. It is the latest nano scale form of carbon to be discovered and is the current hottest topic in material science. Due to its unique electronic properties, rich edge defects and large surface area [5]; it exhibits remarkable electrocatalytic and sensing properties. It is reported that compared to single wall carbon nano tubes, graphene exhibits 60 times more conductivity, better sensitivity and stability, greater sp^2 character and possesses more surface negative charge density [6].

Different methods have been developed for the synthesis of GR, such as micromechanical cleavage, chemical vapor deposition, solvothermal method and chemical synthesis [7]. Graphene is used in optoelectronic devices [8], supercapacitors [9], gas sensors [10], pH sensors [11], and chemical sensors [12] and also many graphene based electrode materials and nano composites have been developed for sensing biomolecules like glucose [13, 14] nicotinamide adenine dinucleotide [15], dopamine [16, 17], phenolic compounds [18], uric acid [17], ascorbic acid [17], catechol [19], epinephrine [20], adenosine-5'-triphosphate [21], bisphenol [22], salbutamol [23], H_2O_2 [24], organophosphorus and carbamate pesticides [25], guanosine-5'-triphosphate [26], kojic acid [27], cocaine [28], some amino acids [29], guanine [30], adenine [30], metronidazole [31] and paracetamol [32].

In this study, Ni^{2+} was incorporated into the graphene oxide film through the electrostatic interactions with negatively charged graphene. The introduction of graphene facilitates the electron transfer of

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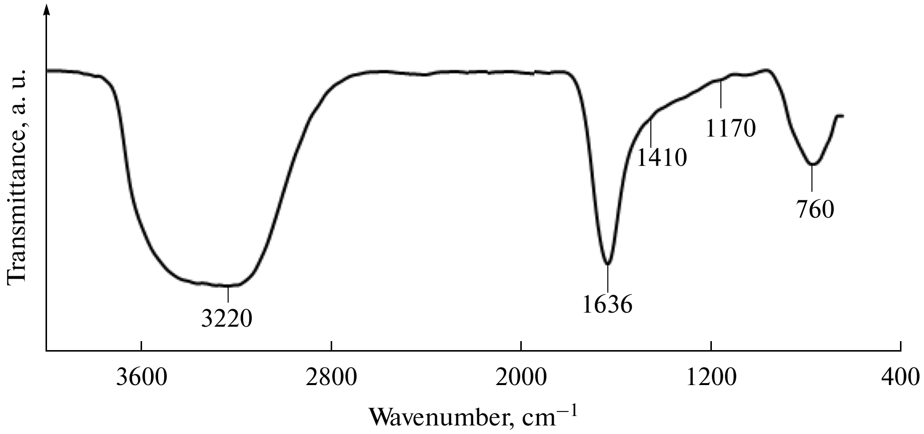

Fig. 1. FT-IR spectra of synthesized GO.

Ni^{2+}. The Ni^{2+}/graphene modified glassy carbon electrode showed good sensitivity and stability for the voltammetric determination of glucose. Moreover, the Ni^{2+}/graphene modified glassy carbon electrode could examine glucose in real samples with good results.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

Graphite flakes were purchased from Asbury Graphite Mills, Inc. (Asbury, N J). Nickel(II) nitrate was purchased from Aldrich. Glucose was of analytical grade from Merck. The concentration of glucose was adjusted by injection with a syringe of the required volume of 0.01 M glucose in the 0.1 M NaOH solution. All other chemicals were analytical grade and were used as purchased.

Electrochemical measurements were performed with an Autolab Potentiostat/Galvanostat (Netherlands). The three-electrode system consists of a Ni/GR/GCE as working electrode, Ag|AgCl|KCl (3 M) as the reference electrode and a platinum wire as an auxiliary electrode (Metrohm) were used in all voltammetric experiments. LEO-Libra 120 microscope was employed for TiO_2-NPs transmission electron microscopy (TEM) images. Fourier transform infrared spectroscopy (FT-IR) studies were carried out with BRUKER FT-IR spectrometer.

2.2. Graphene Oxide Synthesis

Graphene oxide was fabricated based on the reported procedure [33]. Graphite oxide was obtained by oxidation of 1 g of graphite flakes with 120 mL of H_2SO_4 (60%), 13 mL of H_3PO_4 (60%) and 6 g of $KMnO_4$. The mixture was stirred for three days to complete oxidation of the graphite. During oxidation, the color of the mixture changed from dark purplish-green to dark brown. To stop the oxidation process, 7 mL of H_2O_2 (30%) solution and 150 g of ice were added and the color of the mixture changed to bright yellow, indicating a high oxidation level of graphite. The formed graphite oxide was washed three times with 1.0 M HCl solution and repeatedly with deionized water until a pH of 4.0–5.0 was achieved. The washing process was carried out using a simple decantation of the supernatant with a centrifugation technique. During the washing process with deionized water, the graphite oxide underwent exfoliation, which resulted in thickening of the graphene oxide solution, forming graphene oxide gel.

2.3. Characterization of Graphene Oxide

The FT-IR spectra of GO (Fig. 1) shows a broad band at 3320 cm^{-1}, which was related to the OH groups, and band at 1637 cm^{-1}, which is typical of carboxyl and carbonyl groups [34]. Besides, other C–O functionalities such as C–OH (1410 cm^{-1}) and C–O–C (1170 cm^{-1}) are also clearly visible [35]. Furthermore, band at 760 cm^{-1} is because of epoxy symmetrical ring deformation [36]. These main characteristic peaks indicated that GO had been synthesized successfully.

The morphology and structure of the GO were further observed by TEM. The overall view shown in Fig. 2a clearly provided more detailed morphological information for GO. It exhibited that the typical wrinkle morphology of GO was exfoliated into single or very thin layers. The crumpled structure of the nanosheet exhibited in TEM images maybe due to the multiplicity of oxygen functionalities in GO thin layers [37]. The Ni/GO image (Fig. 2b) indicates a random distribution of Ni^{2+} ions at graphene oxide surface.

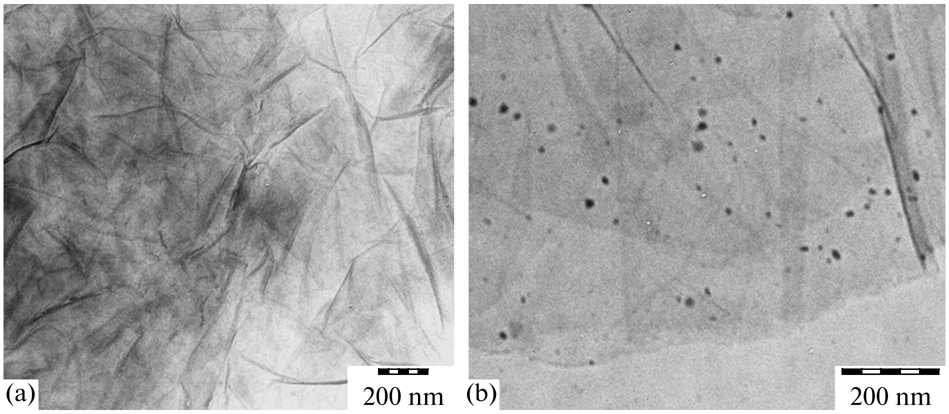

Fig. 2. TEM images of (a) synthesized GO and (b) Ni/GO.

2.4. Preparation of Ni/GR/GCE

The as-prepared graphene contains a variety of oxygen functionalities (–OH, –O–, and –COOH) and is negatively charged when dispersed in water [38]. The presence of negatively charged groups and the conductivity of graphene make it attractive matrix for the immobilization of positively charged molecules in electrochemistry, such as Ni^{2+}. Prior to modification, the bare GCE was polished on chamois leather with 0.05 μm alumina powder. Then it was thoroughly sonicated in deionized water and absolute ethanol, respectively. 1 mL of synthesized graphene oxide gel was dispersed in 5 mL water with the aid of ultrasonic agitation. The cleaned GCE was coated by casting 5 μL of the GO solution and dried at 50°C in an oven air to remove the solvent. Then the GO/GCE was immersed in 1.0 mM nickel nitrate solution for 10 min to obtain Ni/GO/GCE. The Ni/GR/GCE was prepared with cyclic voltammetric scanning of Ni/GO/GCE in the potential range from –1.5 to 0.2 V in 0.1 M phosphate buffer solution at 50 mV s^{-1} for 5 cycles.

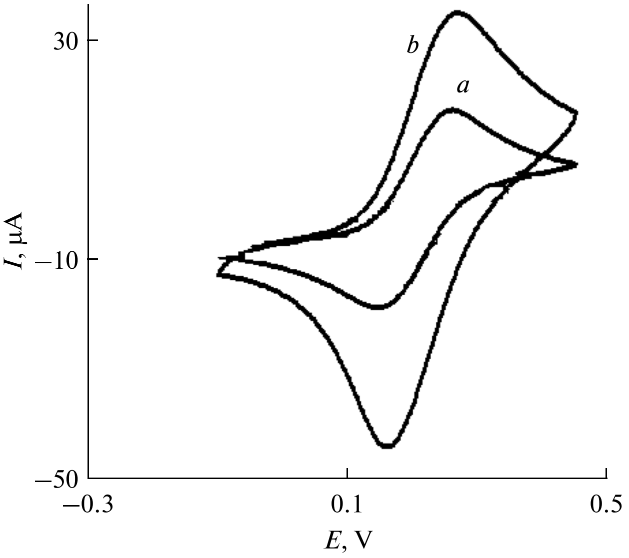

Fig. 3. The cyclic voltammogram of (*a*) GC and (*b*) GR/GC electrodes in 1.0 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl as supporting electrolyte at a scan rate 50 mV s^{-1}.

3. RESULTS AND DISCUSSIONS

3.1. A Characterization of Ni/GR/GCE

CV and EIS were used to characterize the modification of the electrode in 1.0 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl solution. Figure 3 compares the CV responses at GC and GR/GC electrodes in the above solution, respectively. As shown, after modification of GC electrode with GR film, the anodic peak and cathodic peak current is increased, indicating GR can improve the electroactive surface area of electrode (curve *b*) due to the formation of conduction pathway in the GR film.

EIS was employed to further investigate the impedance changes of the electrode surface in the modified process. Figure 4 shows the results of the EIS of bare GCE, GR/GCE in the presence of 1.0 mM $Fe(CN)_6^{3-/4-}$. To understand clearly the electrical properties of the as prepared electrodes/solution interfaces, the semicircle diameter equals the charge transfer resistance (R_{ct}). This resistance exhibits the electron transfer kinetics of the redox-probe at the electrode interface. As shown in Fig. 5, there is charge transfer resistance about 2.71×10^3 Ohm for $Fe(CN)_6^{3-/4-}$ at bare GCE (curve *a*). After modifying GCE with GR film, the R_{ct} decreased to about 1.01×10^3 Ohm (curve *b*), indicating that the GR film is a

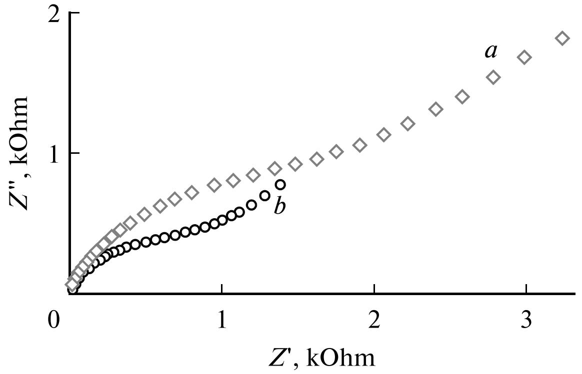

Fig. 4. Impedance plots of bare (*a*) GCE and (*b*) GR/GCE in the presence of 1.0 mM $\mathrm{Fe(CN)_6^{3-/4-}}$ containing 0.1 M KCl as supporting electrolyte.

electron conducting tunnel with a large surface area, which makes it easier for the electron transfer to take place. These data showed that the GR film has been successfully attached to the electrode surface and formed a tunable kinetic barrier.

3.2. Electrocatalytic Behavior of Ni/GR/GCE

The electrocatalytic properties of the Ni/GR/GCE toward glucose oxidation in 0.10 M NaOH solution was studied using cyclic voltammetry with increase of glucose concentration. As shown in Fig. 5, at the in 0.10 M NaOH, the anodic peak current at around 450 mV increases with increase of glucose concentration, demonstrating a typical electrocatalytic oxidation process of glucose.

As it is seen from Fig. 6, cyclic voltammograms obtained for bare GCE, GR/GCE and Ni/GR/GCE in 0.1 M NaOH in the presence of 1500 μM glucose. The oxidation of glucose at GCE (curve *a*), GR/GCE (curve *b*) requires very high positive potentials, leading to a poorly defined anodic wave involving very slow electrode kinetics. In contrast, oxidation of glucose at the Ni/GR/GCE in 0.1 M NaOH occurred at much less positive potentials with an increase in the oxidation peak current and the decrease of the reduction peak (curve *c*).

The glucose oxidation on Ni/GR/GCE was explored for amperometric responses of different concentrations of glucose at Ni/GR/GCE with an applied potential of 500 mV in 0.1 M NaOH (Fig. 7). We use the current value to plot with the concentration of glucose (inset). There was a linear relation of the current with concentration of glucose between 10 to 2700 μM. Detection limit is 5.0 μM of glucose with the signal to noise ratio of three.

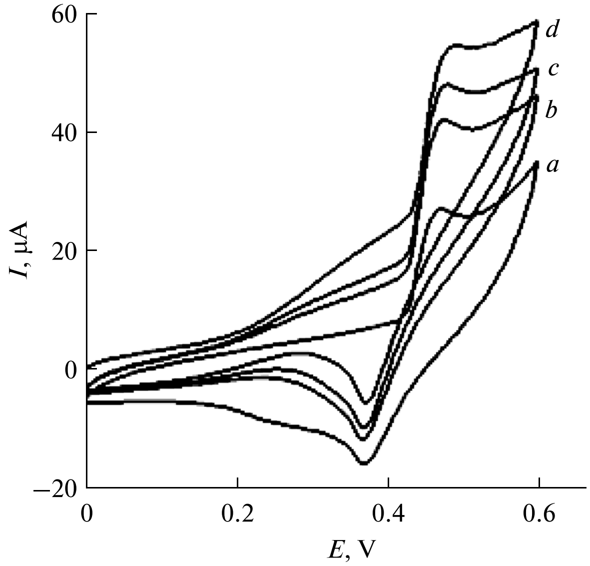

Fig. 5. Cyclic voltammograms of Ni/GR/GCE in the presence of (*a*) 50, (*b*) 150, (*c*) 500 and (*d*) 1000 μM of glucose in 0.1 M NaOH solution at scan rate of 50 mV s^{-1}.

3.3. Reproducibility and Stability of Ni/GR/GCE

The reproducibility and storage stability of the modified electrode were also examined. The relative standard deviation (RSD) of the sensor response to 1.0 mM glucose was 1.9–4.8% for 5 successive measurements. The RSD for detection of 1.0 mM glucose with four sensors prepared under the same conditions was 5.7–7.1%. When the sensor was stored dry and measured at intervals of 1 week, it retained about 84% of its original sensitivity after 4 weeks.

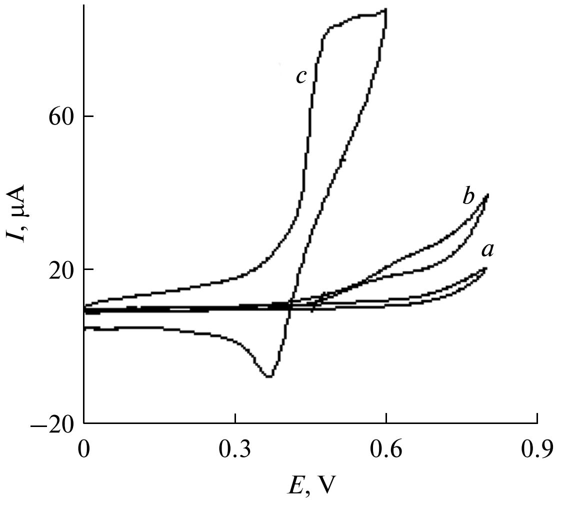

Fig. 6. Cyclic voltammograms of (*a*) GCE, (*b*) GR/GCE and (*c*) Ni/GR/GCE in the presence of 1500 μM of glucose in 0.1 M NaOH solution at scan rate of 50 mV s^{-1}.

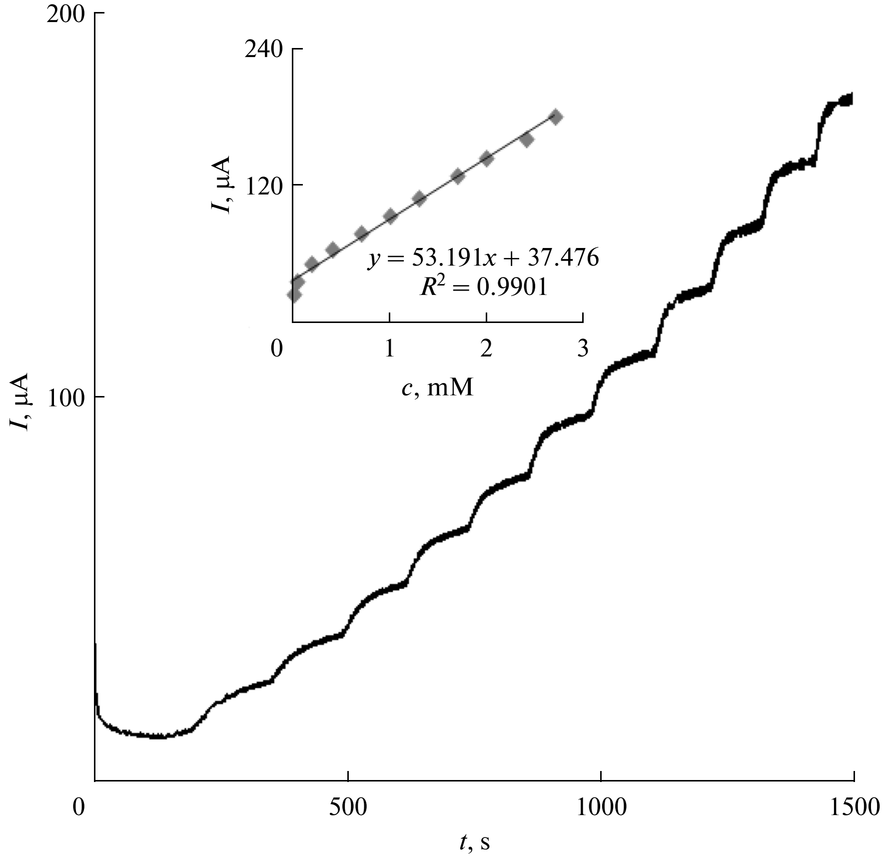

Fig. 7. Current–time curve of Ni/GR/GCE with successive addition of glucose to a stirred 0.1 M NaOH solution. The inset is the calibration curve.

3.4. Determination of Glucose in Human Blood Serum and Analytical Recoveries of Glucose

The response of the modified sensor to the glucose in human blood serum was investigated. The serum sample obtained from hospitalized patient was analyzed. The results were matched with referenced value obtained by the automated standard colorimetric technique in the hospital. Table shows analytical recoveries of the glucose, glucose solutions added to 0.1 M NaOH solution, suggesting the good accuracy of the method.

Assay of glucose in human blood serum samples and recovery of glucose in 0.1 M NaOH solution spiked with different concentrations

Sample	Spiked, mM	Biochemical analyzerin a local hospital, mM	Found, mM	Mean recovery (n = 3)
Blood serum	–	4.70	4.63	–
Glucose	0.1	–	0.09	90 ± 3
	0.30	–	0.31	103 ± 5
	0.15	–	0.14	93 ± 4

CONCLUSIONS

A sensor towards the glucose detection is fabricated by immobilization of Ni(II) onto the GO film via immersing method. The introduction of graphene facilitates the electron transfer of Ni^{2+}. The Ni/GR film modified glassy carbon electrode showed good sensitivity and stability for the voltammetric determination of glucose. The sensor shows a quite wide linear range as well as its experimental limit of detection can be achieved as low as μM. All these advantageous can make the designed sensor applicable in medical, food or other areas. Moreover, the investigation also exhibits that the Ni/GR may be applied as a novel immobilization material for a variety of sensor designs.

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