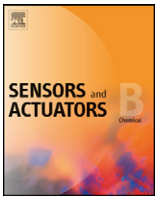
Sensors and Actuators B 174 (2012) 285–291

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb



Electrochemical determination of serotonin on glassy carbon electrode modified with various graphene nanomaterials

Seul Ki Kim, Daekun Kim, Seungwon Jeon*

Department of Chemistry and Institute of Basic Science, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 30 March 2012
Received in revised form 23 July 2012
Accepted 17 August 2012
Available online 25 August 2012

Keywords:
Biosensor
Graphene
Serotonin
Electrochemical determination
Carbon electrode
Amperometry

ABSTRACT

Graphene nanosheets can be produced from easily available starting materials. We synthesized several chemically different types of graphene nanosheet for use as electrocatalysts and characterized their electrochemical properties. We evaluated the surface morphologies of the graphene nanosheets via X-ray photoelectron spectroscopy (XPS) and field emission scanning electron microscopy (SEM), and examined their electrocatalytic activities using electrochemical impedance spectroscopy (EIS). The results were compared with those for other graphene nanosheets, and the efficiency for the electrochemical detection of serotonin, known as an important neurotransmitter, was also studied. Electrocatalytic activities were verified by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and chronoamperometry (CA). A wide variation of results was observed for each of the graphene nanosheets prepared by using three different reductants. Among these graphene nanosheets, RGO1, that was reduced by hydrazine and ammonia solution as a reductant, exhibited high sensitivity, good selectivity, low detection limit, fast response time, and stability.

1. Introduction

Recently, nanomaterial chemistry has undergone significant development. Carbon nanotubes and graphene have received tremendous attention because of their extraordinary properties [1–3]. Graphene is composed of planar sheets of sp^2 bonded carbon atoms. The most common approach to graphite exfoliation is the use of a strong oxidant for obtaining graphene oxide (GO) as non-conductive hydrophilic carbon material [4–7]. Although the exact structure of GO is difficult to confirm, it is clear that in GO, the previously contiguous aromatic lattice of graphene is interrupted by epoxide, alcohol, ketone carbonyl, and carboxylic groups [8–10]. In 1958, Hummers' reported the method most commonly used today. The bulk-scale production of individual graphene sheets is still challenging although several methodologies have been developed. The proposed mechanism for the removal of epoxide and hydroxyl groups from GO will facilitate exploitation of the novel strategies for graphene preparation. A rapid and cost-effective approach to the reduction of GO using hydrazine [11] or hydroxylamine [12] as a reductant has been reported. Since graphene was discovered in 2004, it has emerged as a promising candidate for generating novel hybrid materials with excellent properties for a wide variety of potential applications in catalyst support [13,14], electronic components [15,16], chemical sensor [17–20], and Li ion batteries [21–23].

Serotonin (5-hydroxytryptamine, 5-HT) is an important biomolecule in physiological systems, playing a vital role in the regulation of mood, sleep, emesis, sexuality, and appetite. Low levels of 5-HT are associated with several disorders, including depression, anxiety, and migraines [24,25]. Extremely high levels of 5-HT can manifest toxicity and potentially fatal effects known as serotonin syndrome [26]. It is well known that different 5-HT receptors can influence each other in their respective release, given their coexistence in biological systems [27]. Thus, research interest has focused on developing electrodes for electrochemical determination of 5-HT. The determination of 5-HT is often complicated by the presence of dopamine (DA), since the oxidation potential of 5-HT (0.38 V) is close to that of DA (0.22 V). Effective measurement of 5-HT levels is of greater value because of their coexistence within biological systems. Several major problems are frequently encountered in the determination of 5-HT concentrations. One is the interference of ascorbic acid (AA), which has a similar oxidation potential and is usually present in vivo at 0.2 mM concentrations. Another is that the voltammetric responses of DA and 5-HT interfere with each other. Otherwise, sensitivity is a key to the detection of 5-HT. These facts have encouraged chemists to develop faster, simpler, and more sensitive techniques to meet the various demands and, as such, many works have been published describing the measurement of serum 5-HT concentrations using chemically modified electrodes [28,29]. Additionally, the importance of the

---

* Corresponding author. Tel.: +82 625303380; fax: +82 625303389.
E-mail address: swjeon3380@naver.com (S. Jeon).

http://dx.doi.org/10.1016/j.snb.2012.08.034

5-HT levels under usual circumstances requires a simple and cost-effective method for its rapid monitoring. Electroanalytical methods meet the above requirements, but normally have low sensitivity with conventional bare electrodes. However, the low sensitivity and poor selectivity associated with electrochemical methods can be overcome by electrode modification. Graphene [17,30], carbon nanotubes (CNTs) [31], and fullerene-modified electrodes [32] have been used by several research groups for simple, fast, and sensitive voltammetric determination of various compounds of physiological importance.

In this work, we studied an electrochemical biosensor for detection of 5-HT in the presence of DA and AA by various types of graphene obtained from different reductants and several methods. The various graphene sheets were characterized with X-ray photoelectron spectroscopy (XPS), field emission scanning electron microscope (FE-SEM), and electrochemical impedance spectroscopy (EIS) measurements. Also, their electrocatalytic behaviors for oxidation of 5-HT were evaluated by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and chronoamperometry (CA) in order to evaluate a suitable modified electrode for 5-HT detection.

2. Experimental details

2.1. Apparatus

A three-electrode assembled cell was employed, consisting of a modified GCE (3.0 mm diameter) as the working electrode, a platinum-wire electrode as an auxiliary electrode and an Ag/AgCl (3.0 M NaCl) electrode supplied by BAS (Model MF-2052) as the reference electrode. All potentials were reported with respect to the Ag/AgCl electrode at room temperature and under an argon atmosphere. Electrochemical techniques, including CV, DPV, and CA, were performed using a BAS 100B/W voltammetric analyzer (Bioanalytical Systems, West Lafayette, IN, USA) in a grounded Faraday cage. EIS was carried out with a model VersaSTAT 3 electrochemical system (Princeton Applied Research, USA). The pH measurements were performed by a pH glass electrode with a JENCO meter. FE-SEM images of the modified electrode were obtained by a JSM-600 field emission scanning electron microanalyzer. The XPS analysis was performed using a VGmultilab 2000 spectrometer (ThermoVG Scientific, Southend-on-Sea, Essex, UK) in an ultra high vacuum. This system uses an unmonochromatized Mg K (1253.6 eV) source and a spherical section analyzer. Survey scan data were collected using a pass energy of 50 eV. Prior to measurement, the sample was treated with a mixture of HNO_3, HF, and HBO_3 in order to affect complete dissolution.

2.2. Chemicals and reagents

All graphite powders (~325 μm flakes), $KMnO_4$, hydrazine monohydrate (N_2H_4, 98%), ammonium hydroxide solution (ammonia solution), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$), NaSH, and 1,4-butanedithiol (SH-$(CH_2)_4$-SH) were obtained from Sigma–Aldrich. 5-HT, DA, and AA were also purchased from Aldrich. All other reagents were of analytical grade and were used without further purification. All electrochemical experiments were carried out at room temperature. The pH of phosphate-buffered saline (PBS) was adjusted with 0.1 M H_3PO_4 and 0.1 M NaOH. High-purity argon was used for deaeration. Doubly distilled water with a resistivity over 18 MΩ cm in a quartz apparatus was used to prepare all aqueous electrolyte solutions. The solutions of 5-HT, DA, and AA were prepared by adding the required volumes of PBS stock solution.

2.3. Preparation of graphene nanosheets

See Scheme 1.

2.3.1. Graphene oxide (GO)

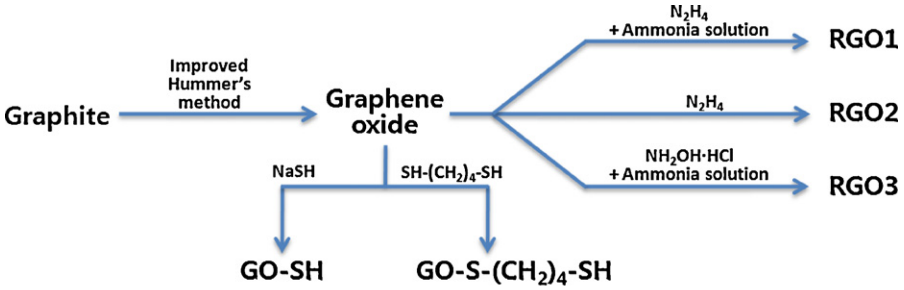
GO was obtained by oxidizing graphite using an improved method [33]. Briefly, a mixture of concentrated H_2SO_4/H_3PO_4 (360:40 mL) was added to a mixture of graphite/$KMnO_4$ (3:18 g) at 50 °C and stirred for 12 h. The reaction was cooled to room temperature and transferred onto ice with 30% H_2O_2 (3 mL). The obtained solution was centrifuged, and then filtered. The solid material was then washed with water, 30% HCl, and finally washed twice with 200 mL of ethanol.

2.3.2. Reduced graphene oxide (RGO) [11]

We synthesized 3 kinds of RGO using different reductants: hydrazine together with ammonia solution (RGO1), only hydrazine (RGO2), and hydroxyl amine together with ammonia solution (RGO3). A colloidal suspension of graphene oxide platelets in purified water (150 mg/50 mL) was prepared by sonication for 3 h. The suspension of RGO1 was subsequently added to 50 μL of hydrazine solution (98%) with 200 μL of ammonia solution (30% in water). In the case of RGO2 and RGO3, the suspension was added to 50 μL of hydrazine solution and 150 mg of hydroxyl amine together with 200 μL of ammonia solution (30% in water). All three formulations were refluxed at 90 °C for 12 h in a heating mantle and cooled to room temperature. Subsequently solutions were centrifuged, and RGO precipitates were washed with deionized water and then dried at 60 °C in a vacuum oven for 24 h.

2.3.3. Thiolated graphene oxide

Four segments of GO and linker materials were separately dispersed together in tetrahydrofuran (THF) into four separate round-bottom flasks and ultrasonicated for 1 h before being stirred at 50 °C for 12 h. The resulting black materials were separated from the mixture by filtration, washed several times with THF and distilled water, and dried in a vacuum oven at 40 °C for 24 h. Therefore, GO treated with NaSH and HS-$(CH_2)_4$-SH became GO-SH and



Scheme 1. The proposed reaction pathway of various types of graphene.