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Highly sensitive and selective dopamine biosensor based on 3,4,9,10-perylene tetracarboxylic acid functionalized graphene sheets/multi-wall carbon nanotubes/ionic liquid composite film modified electrode

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ABSTRACT

A sensitive and selective electrochemical sensor for determination of dopamine (DA) was fabricated based on 3,4,9,10-perylene tetracarboxylic acid functionalized graphene sheets, multi-wall carbon nanotubes and ionic liquid modified glass carbon electrode and the properties of modified electrode were characterized by scanning electron microscopy, transmission electron microscope and electrochemical impedance spectroscopy. The modified electrode showed excellent electrocatalytic activity toward the oxidation of DA. Meanwhile, a possible reaction mechanism related to the oxidation of DA was proposed. The differential pulse voltammetry was used for the determination of DA in the presence of 500 μM ascorbic acid and 330 μM uric acid under the optimum conditions and a good linear relationship between peak current and the concentration of DA was obtained in the concentration range from 0.03 μM to 3.82 mM with a detection limit of 1.2×10^{-9} M (S/N=3). Moreover, the proposed method was successfully applied to determine DA in real sample and satisfactory results were obtained. The results showed that the modified electrode exhibits an excellent catalytic activity, good sensitivity, reproducibility and long-term stability.

1. Introduction

Sensitive, selective, rapid, and cost-effective analysis of biomolecules is important in clinical diagnostics and treatment. Carbon nanostructures, such as carbon nanotubes, carbon nanodots, and carbon nanofibers have been used for this purpose (Liu et al., 2008a; Chen et al., 2007; Cao et al., 2007; Fu et al., 2007; Hao et al., 2007). Recently, graphene-based nanomaterials have attracted great attention for both fundamental science and applied research. Graphene is a two-dimensional sheet of carbon atoms bonded by sp^2 hybrid orbitals. This structural characteristic is the reasons for the extraordinary properties of graphene, which include a very large surface area, high electrical conductivity, exceptional thermal and mechanical properties (Geim and Novoselov, 2007). Because of their novel properties, graphene sheets (GS) have received considerable interest for potential applications in many fields, such as nanocomposites, field-effect transistors, biosensors, and so on. It is noted that GS, which have a high specific surface area, tend to form irreversible agglomerates or even restack to form graphite through strong π–π stacking and van der Waals interaction (Li et al., 2008). Hence the prevention of aggregation is a key challenge in the synthesis and processing of bulk-quantity GS. Recently, it has been found that the functionalization is an efficient method to improve their dispersibility and solubility in aqueous solvent (Zhang et al., 2011; Liu et al., 2010; Li et al., 2009; Hong et al., 2010). It is well known that carbon nanotubes(CNTs) are suitable materials for electrode modification because of the high accessible surface area, low electrical resistance, extremely high mechanical strength and stiffness, outstanding charge-transfer characteristics and high chemical stability (Fang et al., 2009; Guldi et al., 2005; Dai et al., 2006; Rodney et al., 2002). The application of CNTs in the fields of electroanalysis and electrochemical biosensors had been reviewed. Generally, CNTs can enhance the detection sensitivity and improve reversibility as it can promote electron transfer.

On the other hand, ionic liquid (IL) have also received great attention in the last few years due to their excellent properties such as high ionic conductivity, good chemical and thermal stability, negligible vapor pressure, wide electrochemical windows and well biocompatibility. IL can be used as not only the supporting electrolyte but also the modifier for the chemically modified electrodes. The reviews about the application of IL in the

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fields of analytical chemistry or electrochemistry had been reported (Pandey, 2006; Sun et al., 2007). Several groups had used different kinds of IL in electrode modification for the design of new electrochemical sensors or as novel electrocatalytic materials. The studies demonstrated that the presence of IL not only acted as a suitable charge-transfer bridge to facilitate the electrode transfer rate but also exhibited excellent electrocatalytic ability and good anti-fouling ability (Sun et al., 2009a,b; Shul et al., 2006; Choi et al., 2009).

Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system. Low levels of DA may cause neurological disorders such as schizophrenia and Parkinson's disease (Liu et al., 2005; Mo and Ogorevc, 2001). The accurate and rapid determination of DA concentration in body fluid is a significant issue to conveniently trace and diagnose the diseases. Ascorbic acid (AA) is well known for its antioxidant property and usually coexists with DA in biological samples, which concentration is several orders of magnitude higher than that of DA, and have a similar oxidation potential (Yen et al., 2002; Ali et al., 2007; Tang et al., 2008). Therefore, it is of importance to develop a rapid, simple, sensitive sensor for the detection of DA without interference by AA. Recently, different kinds of DA sensors have been fabricated based on chemical modified electrodes, such as polymer electrodes, self-assembled monolayer and carbon nanomaterial film electrodes (Codognoto et al., 2007; Lakshmi et al., 2009; Rodriguez et al., 2008; Kalimuthu and Abraham, 2010; Liu et al., 2008b; Zhu et al., 2009; Kim et al., 2010; Thiagarajan et al., 2009). Graphene and CNTs have widely been used as ideal electrode modified nanomaterials because they can provide more active sites and are easier to fabricate. Several groups had used functionalized graphene, CNTs or negatively charged polymer films to modify electrodes to determination positively charged DA and to eliminate the interference of AA (Hou et al., 2010; Deng et al., 2009; Zhao et al., 2005; Wang et al., 2009; Balamurugan and Chen, 2007). Although the electrochemical responses have been continuously improved, the development of more reliable and efficient sensors for sensitive analysis of DA still remains very challenging. Therefore, the choice of material is essential and important to construct sensors with excellent performances.

In this paper, 3,4,9,10-perylene tetracarboxylic acid functionalized graphene sheets (PTCA-GS) were prepared by a simple synthetic method. The modification of PTCA onto graphene sheets can not only separate the graphene sheets and maintain inherent electronic structure of graphene sheets but also provide a negatively-charged –COOH, which made the resulting PTCA-GS composites disperse well in solvents and provide more active sites. Meanwhile, by combining the advantages of PTCA-GS, MCNT and IL, a novel and more sensitive dopamine electrochemical sensor was fabricated with PTCA-GS/MCNT/IL modified electrodes. Because of synergetic effects of conductive functionalized graphene, MCNT and biocompatible IL, the direct electron transfer between DA and electrode surface can be efficiently achieved. Most importantly, such modified electrode shows good electrocatalytic performance to DA with high sensitivity and good stability. The sensitivity of the sensor along with its improved selectivity might allow for its potential use in the investigation and diagnosis of dopamine-related disease.

2. Materials and methods

2.1. Reagents

Natural graphite powder ($<20\ \mu m$) was purchased from Tianjin Guangfu Research Institute (Tianjin, china). Multi-walled carbon nanotubes (purity $>95\%$) was purchased from Shenzhen Nanotech Port Company (Shenzhen, China). [BMIM][BF_4] was purchased from Lanzhou Institute of Chemical Physics (Lanzhou, China). 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA, 97%), dopamine, ascorbic acid and uric acid were ordered from Sigma-Aldrich (USA). The 3,4,9,10-perylene tetracarboxylic acid (PTCA) solution was made by hydrolyzing 3,4,9,10-perylenete-tracarboxylic dianhydride (PTCDA) in an appropriate amount of 1.0 M sodium hydroxide. Phosphate buffer solution (PBS) was prepared by mixing the stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 and adjusting the pH with 0.1 M H_3PO_4 or 0.1 M NaOH. Unless otherwise stated, other reagents were of analytical grade and were used as received. All aqueous solutions were prepared with double distilled water.

2.2. Apparatus

Electrochemical experiments were performed on a CHI-832 electrochemical workstation (CH Instruments, Shanghai Chenhua Instrument Corporation, China). A three-electrode cell was employed, consisting of a glassy carbon electrode (GCE) or a modified GCE as a working electrode, saturated calomel electrode (SCE) and platinum sheet respectively as the reference and auxiliary electrode. All potentials are referred to the SCE. The electrochemical impedance spectroscopy measurements were performed on a VMP2 multichannel potentiostats. Scanning electron microscopy (SEM) images were determined with a JSM-6701 F field emission scanning electron microscope (Japanese Electron Optics Company). Transmission electron microscope (TEM) images were obtained with a JEM 1200EX transmission electron microscope opened at an accelerating voltage of 80.0 KV (Japanese Electron Optics Company).

2.3. Synthesis of graphene and PTCA-functionalized graphene sheets

Synthesis of graphene: graphite oxide (GO) was synthesized from graphite powder by a modified Hummers method. GS were prepared by the chemical reduction of GO with hydrazine. Typically, GO (100 mg) was dispersed in 100 mL of deionized water and the dispersion was sonicated using a KQ-250DE ultrasonic bath cleaner until it became clear with no visible particulate matter. Hydrazine monohydrate (1 mL) was then added to the dispersion and the mixture was heated in an oil bath at 100 °C for 24 h. After the completion of the reaction, the reduced graphite oxide was collected by filtration, followed by washing with deionized water several times to remove excess hydrazine. The final product was dried in a vacuum oven at 80 °C for 24 h.

Synthesis of PTCA-GS: PTCA-GS was prepared by a simple synthetic method. Typically, PTCA (7.4 mg) and GO (30.0 mg) were dispersed in 25.0 mL water and then stirred at 40 °C for 24 h. Subsequently, hydrazine solution (0.5 mL) and ammonia solution (0.5 mL) were added to the mixture and the reaction mixture was held at 90 °C for 50 min under vigorous agitation. Finally, the precipitation was filtered, washed with deionized water for five times, and dried in a vacuum oven at 25°C.

2.4. Fabrication of PTCA-GS/MCNT/IL, MCNT/IL and PTCA-GS/IL modified electrodes

Before the modification, GCE (3 mm) were carefully polished to a mirror with 1.0 μm, 0.3 μm and 0.05 μm alumina slurry in sequence, rinsed thoroughly with doubly distilled water between each polishing step, finally sonicated in 1:1 HNO_3, 1:1 ethanol, and doubly distilled water, and dried under a nitrogen stream. And then the electrode was immersed in 1.0 M H_2SO_4 and treated by cyclic voltammetric scanning in the potential range of -1.0 to $+1.0$ V (vs. saturated calomel electrode, SCE) until a stable CV profile was obtained. For the preparation of PTCA-GS/MCNT/IL/GCE, MCNT/IL/GCE and PTCA-GS/IL/GCE, 10 mL of 0.1 mg mL^{-1} PTCA-GS/MCNT was first mixed with 50 μL of IL and sonicated for 2 h to form a homogenous mixture. Then, 5 μL of the mixture was dropped on the pretreated GCE with a microsyringe and dried for 24 h in air before