Graphene oxide-Ag/poly-L-lysine modified glassy carbon electrode as an electrochemical sensor for the determination of dopamine in the presence of ascorbic acid

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ABSTRACT

The graphene oxide (GO) and Ag hybrid matrix GO-Ag were coated onto the glassy carbon electrode (GCE) surface, then, poly-L-lysine films (PLL) were prepared by electropolymerization with cyclic voltammetry (CV) method to prepare GO-Ag/PLL modified glassy electrode (GO-Ag/PLL/GCE). The electrochemical sensor based on GO-Ag/PLL/GCE was used to sensitively determine dopamine (DA) in the presence of ascorbic acid (AA). Electrochemical behaviors of DA and AA mixture were investigated on GO-Ag/PLL/GCE by CV, which gave a baseline separation of the oxidation peak potential by 232 mV. So the modified electrode was suitable for determine of DA in the presence of AA. The GO-Ag/PLL/GCE electrode improved the DA electrochemical catalytic oxidation, which demonstrates that GO-Ag/PLL/GCE has a remarkable electrocatalytic activity towards the oxidation of DA. Moreover, PLL modified electrodes have good stability, excellent permselectivity, more active sites and strong adherence to electrode surface, which enhanced electrocatalytic activity. Under the optimized conditions, there were linear relationships between the peak currents and the concentrations in the range of 0.1–10 μM for DA, with the limit of detection (LOD) (based on S/N = 3) of 0.03 μM for DA. The GO-Ag/PLL/GCE sensor was successfully applied to the determination of DA in bovine serum samples and showed high selectivity, sensitivity, and reproducibility.

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1. Introduction

Dopamine (DA) is a kind of import catecholamine neurotransmitters in the mammalian central nervous system [1–3]. It implicates many human behaviors for example motivation, motor function, reward, and cognition, and plays an important role in memory and learning [4,5]. Many research studies show that abnormal levels of DA may cause neurological disorder such as senile dementia, HIV infection, schizophrenia and Parkinson's disease [6–8]. So the accurate and rapid determination of DA concentration in body fluid is a significant issue to conveniently trace and diagnose such diseases in clinical practice. However, ascorbic acid (AA) usually coexists with DA in real systems. Therefore, reliable analytical procedures with high sensitivity are required for determination of DA in the presence of AA in various matrices.

So far, many methods have been established for simultaneous determination of DA and AA in their mixture, such as surface plasmon resonance [9], capillary electrophoresis [10], liquid chromatography [11,12], and fluorescence [13]. However, most of the above-mentioned methods are costly and time consuming, complicate pretreatment and

are low in sensitivity. Because AA and DA are electroactive compounds, electrochemical methods are commonly used for their determination in body fluids and pharmaceuticals. Furthermore, electrochemical methods have more advantages such as fast analysis and automation, reduction of costs, high sensitivity and selectivity, wide linear dynamic, low power requirement, and no requirement for previous separation [14–17]. However because of the homogeneous catalytic effect, the similarity of oxidized potentials of DA and AA at traditional electrodes results in overlaps of voltammetric responses, which makes determination of DA in the presence of AA very difficult [18]. To address the problem, modified electrodes have been achieved for separate their signal potentials with enhanced intensity. Recently, many advanced materials including polymers [19,20], metal complexes [21,22], metal oxide [23], multi-walled carbon nanotube [18,24] and graphene-based nanomaterials [23,25] have been reported to effectively detect DA and AA. Among these electrode materials, graphene oxide (GO) has attracted significant interest since experimentally produced in 2004 [26–29]. GO's excellent properties make it suitable for applications in highly sensitive and selective determination [30–32]. It can be used as an excellent electrode material for analytical applications and diagnostic research purpose. Considering the characteristic of the graphene oxide, GO could be used as an electrochemical sensor to study the oxidation of DA. On the other hand, the properties of GO are similar to graphene,

with graphite structures of a single atomic plane and high surface areas. These unique structures could promote target molecules substantially adsorbed on the surface to give a higher current signal [33]. Furthermore, GO has a lot of oxygen-containing functional groups, such as –OH, –COOH and C–O–C, which will hydrogen bonding increase the attachment of DA to the surface of GO by the effective groups ($-$OH,$-$NH$_2$).

Recently, GO-based composite materials with metallic nanoparticles have been intensively studied with regard to potential applications in the area of energy storage, catalysis, and chemical sensors [34–36]. The GO combined with metallic composite materials can improve the hybrids electronic and thermal conductivity [37]. Ag nanoparticles have excellent catalytic capabilities owing to their large active surface area and favorable lattice planes compare to bulk metal. In practical usage, Ag nanoparticles are generally dispersed into a conducting support to avoid their agglomerations and keep their activities [38].

Polymer modified electrodes have been paid increasing attention in electrochemical analysis due to their more active sites, excellent permselectivity, good stability and strong adherence to electrode surface. Poly (L-lysine) (PLL) film can be easily formed on electrode surface by electropolymerization of L-lysine. PLL has been receiving much attention owing to its good biocompatibility, versatility and easiness of the preparation, relatively good solubility in water, a flexible structure framework and a good deal of active amino groups [39,40], which make them suitable for applications in electrochemical determination.

Combining the advantageous features of metal hybrids GO and PLL, we herein present directly GO-Ag complex with PLL as a linker through a covalent amide group for further attaching bioactive molecules for the determination of DA in the present of AA. These materials not only have the inherited advantages from each component material, but also improve properties due to synergetic effect. The results show that the sensitivity of the proposed method is better or comparable than the previous ones. The proposed electrochemical sensor can be used for determination of DA in bovine Serum samples with satisfactory results.

2. Experimental

2.1. Apparatus

The phases of the samples were identified by powder X-ray diffraction (XRD) analysis using a SIEMENS D5005 diffractometer with Cu Kα radiation at 40 kV and 30 mA. Raman spectra were obtained on a J-Y T64000 Raman Spectrometer with 514.5 nm wavelength incident laser light. Transmission electron microscopy (TEM) micrographs were recorded on a Hitachi 600 transmission electron microscopy (Japan) operating at 200 kV. The sample for TEM characterization was prepared by placing a drop of colloidal solution of the sample dispersed in ethanol over a carbon-coated copper grid. Scanning electron microscopy (SEM) images were obtained from HITACHI SU8010 operating at 20.0 kV, equipped to perform elemental chemical analysis by energy dispersive X-ray spectroscopy (EDX). Because the sample has good electrical conductivity, the sample was not metal-coated prior to measurements to eliminate any charging effects for SEM images. Electrochemical measurements were carried out with an Autolab-PGSTAT302 electrochemical workstation (Metrohm). A conventional three-electrode system, including a bare or modified glassy carbon electrode (GCE, 3 mm in diameter) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum (Pt) wire as counter electrode was used in this work. All electrochemical experiments were carried out under highly pure nitrogen at room temperature.

2.2. Reagents

Graphite powder (320 mesh, spectrum pure), L-lysine, DA, AA were purchased from Aladdin Reagent Co (Shanghai, China). AgNO$_3$, H$_2$SO$_4$,

KMnO$_4$, H$_2$O$_2$ (30 wt.%), NaNO$_3$ and N, N-dimethylformamide (DMF) were purchased from Beijing Chemical Reagent Co. (Beijing, China). All chemicals were used as received without further purification. All solutions were freshly prepared with deionized water.

2.3. Synthesis of graphene oxide (GO)

GO was synthesized from graphite powder by a modified Hummers method [41,42]. In a typical synthesis, graphite powder (1 g) and H$_2$SO$_4$ (25 mL, 98 wt.%) were mixed in a 250 mL round-bottom flask and stirred at room temperature for 12 h. Afterwards, of NaNO$_3$ (500 mg) was added into the mixture and stirred at 0 °C for 30 min. Subsequently, KMnO$_4$ (3.65 g) was slowly added to prevent the temperature to exceed 20 °C and the suspension was stirred for 2 h. After that, the temperature of the system was kept at 35 ± 3 °C for 30 min, then, 46 mL of water was slowly added into the suspension within 25 min, and the temperature of the system was increased to 98 °C and maintained for 15 min. At last, 27.5 mL of water and H$_2$O$_2$ (3.5 mL, 30%) were added, followed by water washing and filtration. The as-prepared graphite oxide was exfoliated in water by bath ultrasonication for 2 h, to form GO. Finally, the as purified GO was dispersed well in water (1 mg/mL).

2.4. Synthesis of GO-Ag nanocomposite

The GO-Ag nanocomposite was prepared by reducing silver ions directly on GO with glucose as reducing and stabilizing agent. The typical procedure for GO-Ag nanocomposite synthesis was described as follows [43]. Firstly, GO powder (15.0 mg) was dispersed in water (15.0 mL) by sonication for 1 h to form a stable GO colloid and then glucose was added to this solution under stirring. Second, ammonia (0.55 mol.L^{-1}) was added slowly to a solution of AgNO$_3$ (5.0, 8.0, 10.0, 12.0, and 15.0 mL, respectively, 0.06 mol.L^{-1}) until the AgOH/Ag$_2$O precipitate dissolved to form Ag (NH$_3$)$_2$OH solution. Subsequently, the Ag(NH$_3$)$_2$OH solution was mixed with the GO and glucose-containing solution. After being stirred for 0.5 h, the mixture was kept undisturbed at room temperature for 2 h. The slurry-like product was centrifuged and washed with excess water repeatedly to remove any impurities. Finally, the obtained product was dried overnight in an oven at 60 °C, and then GO-Ag nanocomposites with different Ag loading were obtained. By thermogravimetric analysis (TG), content of Ag in the GO-Ag nanocomposites were 8.5, 13.7, 21.6, 29.6 and 34.8 wt.%, respectively.

2.5. Preparation of GO-Ag/PLL/GCE

A glassy carbon electrode (GCE) was first polished stepwise with 1.0, 0.3 and 0.05 μm alumina powders, and then washed with ethanol-water (1:1, V/V) and double distilled water in an ultrasonic bath for 30 min. Different Ag loading of GO-Ag hybrids materials (5.0 mg) were dispersed in DMF (10 mL) with the aid of ultrasonic bath for 30 min to give a 0.5 mg.mL^{-1} black suspension. Then the prepared suspension (5.0 μL) was coated onto the fresh GCE surface using a micropipette, followed by evaporating the solvent under an infrared lamp. The obtained electrode was denoted as GO-Ag/GCE. The electropolymerization of L-lysine on GO-Ag/GCE was performed by dipping the above GO-Ag/GCE into PBS buffer solution (pH 7.5) containing 1.0×10^{-3} mol/L L-lysine by cyclic potential scanning from -1.0 to 0.4 V for 30 cycles with a scan rate of 100 mV s^{-1}. The final obtained electrode was denoted as GO-Ag/PLL/GCE. The schematic representation of the immobilization and hybridization of DA on the GO-Ag/PLL is shown in Scheme 1.

3. Results and discussion

3.1. Characterizations of GO-Ag nanocomposites

Typical XRD patterns of the as-prepared samples are shown in Fig. 1. After chemical oxidation, it was found that the peak at 26.6° for graphite