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Short communication

Simultaneous voltammetric determination for DA, AA and NO_2^- based on graphene/poly-cyclodextrin/MWCNTs nanocomposite platform

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ABSTRACT

In the present work, graphene sheets (GS) and multiwall carbon nanotubes (MWCNTs) were dispersed in the mixed solution of cyclodextrin (CD) and cyclodextrin prepolymer (pre-CD) and were used as modifier to fabricate chemical modified electrode to simultaneous detect dopamine (DA), ascorbic acid (AA) and nitrite (NO_2^-). CD cross-linked pre-CD (CDP) displays excellent film forming ability, which made the electrode stable. Comparing with CDP–GS, CDP–MWCNTs and CDP–GS–MWCNTs modified electrodes, the CDP–GS–MWCNTs displays higher catalytic activity and selectivity toward the oxidation of DA, AA and NO_2^-, revealing that MWCNTs effectively inhibited the stacking of individual GS and enhanced the utilization of GS based composites. The host–guest chemical reaction ability of CD and π–π stacking interaction between detected molecules and GS–MWCNTs surface were considered as the main reasons of the successfully simultaneous detection of DA, AA and NO_2^-. Cyclic voltammetry (CV), scanning electron microscopy (SEM) and different pulse voltammetry (DPV) were employed to characterize the biosensor. The linear response range for AA, DA and NO_2^- were 5 μM–0.48 mM, 0.15–21.65 μM and 5 μM–6.75 mM, respectively and the detection limits were 1.65 μM, 0.05 μM and 1.65 μM.

1. Introduction

Dopamine (DA) is a neurotransmitter and plays a very important role in the function of central nervous, renal, hormonal and cardiovascular system (Heien et al., 2005). The determination of DA is a subject of great importance for investigating its physiological functions and diagnosing nervous diseases resulting from DA abnormal metabolism, such as epilepsy, senile dementia, Parkinsonism, schizophrenia and HIV infection (Zhou et al., 2010; Ali et al., 2007). However, a major problem of electrochemical detection of DA in real samples is coexistence of many interfering compounds, such as AA and NO_2^-.

Many papers have shown that NO plays a crucial role for intra- and intercellular signally in various tissues. In the central nervous system, NO acts as a neurotransmitter or a neuromodulator (Garthwaite, 1991). Some groups reported that NO enhanced the release of DA, whereas others claimed that NO inhibits DA release (Guevara-Guzmam et al., 1994; Rose et al., 1994). Up to now, the physiological results of NO for DA release in the striatum are controversial. But what is undisputed is that NO can be oxidized to NO_2^- as fast as in a few seconds by reacting with dissolved oxygen in solution and in biological circumstance. From this information we can know that NO_2^- and DA could be coexisted in organisms. AA usually coexists with DA and presents in vivo at concentrations 10^2–10^3 times than DA, and they are oxidized at similar potentials (Alarcon-Angeles et al., 2008). Therefore, it is important to establish simply, rapid and sensitive methods for simultaneous detect DA, AA and NO_2^-. For this purpose, various materials have been employed to modify electrodes, such as nanoparticles, polymers, carbon nanotubes and organic redox mediator (Thiagarajan and Chen, 2007; Nien et al., 2009; Zhang et al., 2005; Palraj Kalimuthu and John, 2009).

Graphene sheets (GS), a two-dimensional (2D) carbon material, have high surface area, superior conductivity and broad potential window (Stankovich et al., 2006; Novoselov et al., 2004; Geim and Novoselov, 2007). Due to its unique properties, graphene has been considered as attractive new material in the design of novel sensors. However, owing to the π–π interaction between individual GS, the GS easily tend to form irreversible agglomerates or even restock to form graphite through Van der waals interactions (Si and Samulski, 2008). Since the aggregated of GS are similar to graphite, the expected performances of GS will lose significantly. In order to improve the solvency and dispersibility of GS, many methods have been reported, such as chemical modification, acid oxidation and non-covalent functionalizations (Sundaram et al., 2008; Bekyarova et al., 2009; Wu et al., 2010). However, these methods are complex, difficult to control or sever which may damage the structure of GS and lose of the intrinsic properties of GS-based materials. Recently, Yang et al. (2010b) reported a new method to reduce the stacking of GS by introducing 1D carbon nanotubes to form a 3D nanohybrid.

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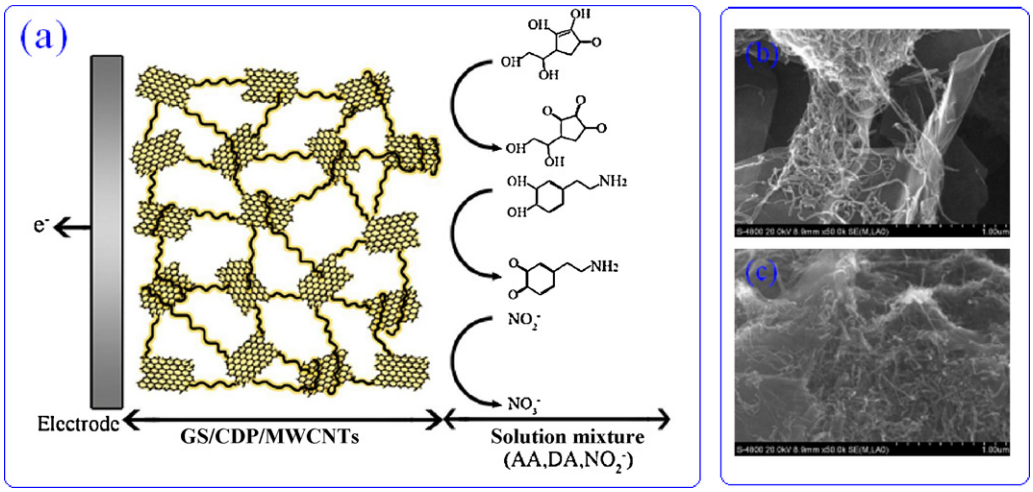

Scheme 1. Schematic representation of the simultaneous electrocatalytic oxidation of AA, DA and NO_2^- by the CDP–GS–MWCNTs modified GCE (a); SEM images of GS/MWCNTs (b) and CDP/GS/MWCNTs (c) nanocomposite.

The long and tortuous MWCNTs bridged adjacent GS and inhibited their aggregation efficiently, which enhanced the utilization of GS-based composites.

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide, consisting of seven glucopyranose unites (Szejtli, 1998). It possesses an electronic and hydrophobic interior microenvironment in its cavity structure, which allows hydrophobic molecules to be easily trapped into its cavity by displacing the water (Breslow and Dong, 1998). And for having a lot of hydroxy groups, the exterior of its cavity is hydrophilic. For these reasons, β-CD has been studied extensively as a model of enzymatic catalysis (Fragoso et al., 2002; Liu et al., 1998), molecular recognition (Ozoemena and Stefan, 2005) and increasing solubility (Badr-Eldin et al., 2008). Alarcon-Angeles et al. (2008) and Tan et al. (2010) have reported using β-CD to detect dopamine (DA) in the presence of ascorbic acid (AA). In their papers, β-CD was adsorbed on the modified electrode physically. Due to the water-solution characteristics of β-CD, these biosensors' stability was not so satisfied. To overcome the problem, water-insoluble polymer is needed. Water-soluble β-CD cross-linked with water-insoluble polymer (pre-CDP) displayed excellent film-forming ability and compared with β-CD modified electrode, the mixture of β-CD and pre-CDP modified electrode more stable (Yang et al., 2010a).

In this work, GS and MWCNTs were dispersed in the mixed solution of β-CD and pre-CDP, and were used as modifiers to fabricate a biosensor to simultaneous detect DA, AA and NO_2^-. The 1D MWCNTs and 2D GS formed a 3D hierarchical structure, and MWCNTs effectively inhibited the stacking of individual GS and enhanced the utilization of GS based composites. β-CD increased the dispersibility and solubility of GS–MWCNTs, and the host–guest recognition of β-CD has high electrochemical sensitivity for the determination of DA and AA. Scheme 1a shows the structure of the modified electrode and explains the electron mediating properties of CDP–GS–MWCNTs nanohybrid film toward the oxidation of AA, DA and NO_2^-.

2. Experimental

2.1. Reagents and materials

MWCNTs (>95% purity) were obtained from Chengdu Organic Chemicals Co. Ltd. of the Chinese Academy of Science and purified by fluxing in concentrated nitric acid for 7 h prior to use. Graphene sheets (GS) were purchased from Chengbo Chemical materials Co. Ltd. (Xian, China). β-Cyclodextrin (β-CD) and epichlorhydrin were obtained from Shanghai Chemical Reagents Co. Ltd. (Shanghai, China). Phosphate buffer solution (PBS) with various pH were prepared by using the stock solution of 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, and the supporting electrolyte was 0.1 M KCl. All chemicals used were of analytical grade and were used as received without further purification. Doubly distilled water was used throughout all experiments.

2.2. Apparatus

Electrochemical measurements were carried out using CHI 660D electrochemical workstation (Shanghai CH instruments Co., China). A three-compartment electrochemical cell contained a modified glassy carbon electrode (GCE, Φ = 4 mm) as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. The surface morphologies of composites were identified by the scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan). All experiments were carried out at room temperature.

2.3. Preparation of β-CD prepolymer (pre-CDP)

The pre-CDP was prepared according to the literature (Ekberg et al., 1989; Yang et al., 2010b) with slightly modification. Briefly, 10.5 g β-CD was dissolved in 25 mL 35% NaOH, and then 8.5 mL epichlorhydrin was added drop by drop with stirring. The reaction solution was heated to 90 °C for 5 min with stirring. After cooling to room temperature, the pH value was adjusted to 7.0 with 6 mol L^{-1} HCl solution, and then was subjected to dialysis (molecular weight cut-off, 3500). The dialysate was dried at 60 °C under vacuum. Thus the pre-CDP was obtained.

2.4. Fabrication of the biosensor

The GCE was polished subsequently with 0.3 and 0.05 μm alumina slurry, and then sonicated in ethanol and deionized water for several minutes and dried in air. 1 mg GS and 1 mg MWCNTs were dispersed 1 mL of mixed solution of β-CD (1 wt.%) and pre-CD (1 wt.%) by sonicated for 1 h to obtained a stable black suspension. 5 μL of the suspension was dropped on the surface of the GCE to fabricate a biosensor (noted CDP–GS–MWCNTs/GCE). For comparison, CDP–GS/GCE and CDP–MWCNTs/GCE were also prepared by dropping 5 μL CDP–GS and 5 μL CDP–MWCNTs mixed solution on the GCE, and dried in air at room temperature.

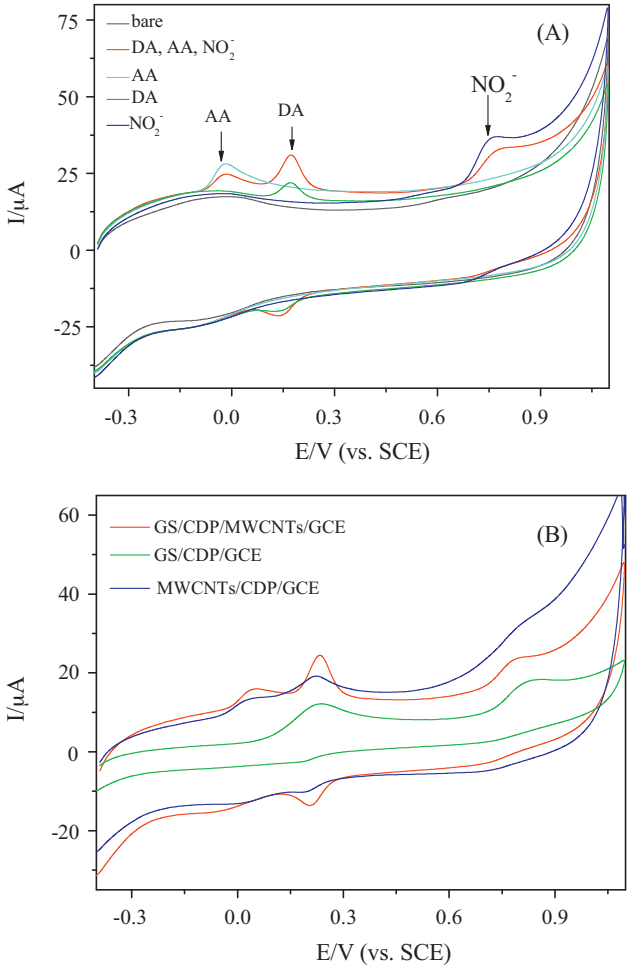

Fig. 1. (A) Cyclic voltammograms of the CDP–GS–MWCNTs modified GCE by adding 50 μM AA (cyan), 5 μM DA (green), 50 μM NO_2^- (blue) and mixture of the three species (red) in 0.1 M pH 6.0 PBS, respectively and (B) cyclic voltammograms of CDP–GS (green), CDP–MWCNTs (blue) and CDP–GS–MWCNTs (red) modified GCEs in 0.1 M pH 6.0 PBS containing 50 μM AA, 5 μM DA and 50 μM NO_2^-, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Structure characterization

The morphologies and microstructures of GS–MWCNTs and CDP–GS–MWCNTs nanohybrids were investigated by means of SEM. As shown in Scheme 1b, the long and tortuous MWCNTs bridged adjacent GS and reduced the aggregation of GS, leading to a highly 3D nanohybrid structure to enhance the utilization of GS. With the addition of β-CD (Scheme 1c), the surface morphologies changed and can be seen that GS and MWCNTs dispersed well, which demonstrated that β-CD increased the dispersibility and solubility of GS–MWCNTs efficiently.

3.2. Cyclic voltammetric behaviors of the modified electrode

Cyclic voltammetric (CV) behaviors of the CDP–GS–MWCNTs modified GCE were investigated by adding AA (cyan), DA (green), NO_2^- (blue) and mixture of the three species (red) in 0.1 M pH 6.0 PBS (Fig. 1A), respectively. The peak of AA, DA and NO_2^- oxidation appeared at −0.06 V, 0.17 V and 0.7 V, respectively. The peak potentials of the mixture of the three species almost occurred at the same potential of AA, DA and NO_2^-,

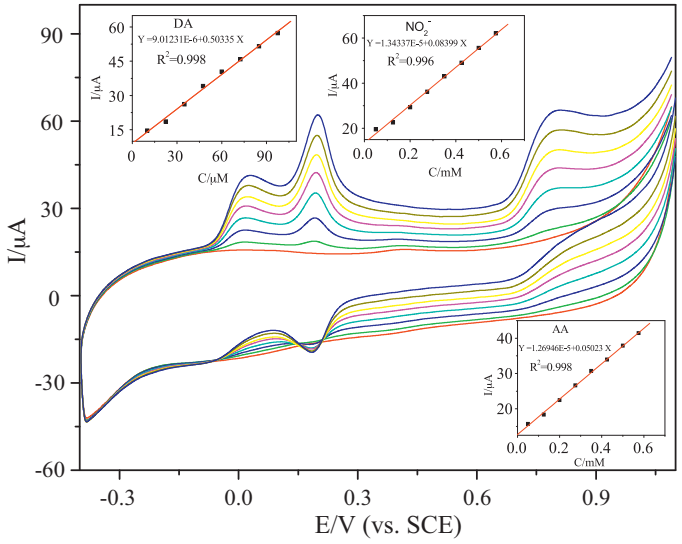

Fig. 2. Cyclic voltammograms of the CDP–GS–MWCNTs modified GCE in 0.1 M pH 6.0 PBS containing individual concentration of AA, DA and NO_2^- mixture. [AA]: 0.0, 0.050, 0.125, 0.200, 0.275, 0.350, 0.425, 0.500, and 0.575 mM. [DA]: 0.0, 10.0, 22.5, 35.0, 47.5, 60.0, 72.5, 85.0, and 97.5 μM. [NO_2^-]: 0.0, 0.050, 0.125, 0.200, 0.275, 0.350, 0.425, 0.500, and 0.575 mM.

which indicated that the discrimination of the three species was feasible.

Fig. 1B compares the CV responses of the mixture of AA, DA and NO_2^- on different modified electrodes. On the CDP–GS/GCE (green), the peak of DA and NO_2^- oxidation were observed at 0.17 V and 0.7 V, but there was no peak at −0.06 V. On the CDP–MWCNTs/GCE (blue), three peaks appeared at −0.06 V, 0.17 V and 0.7 V, respectively, but its anodic peak currents were unconspicuous and smaller than that on the CDP–GS–MWCNTs/GCE (red), suggesting that the adding of MWCNTs, not only decreased the interfacial resistance, but also increased the polymer contact area and enhanced the electrochemical properties of the modified electrode. The reasons may be ascribed as follows: (i) MWCNTs effectively inhibited the stacking of individual GS and enhanced the utilization of GS based composites and (ii) not removed metal nanoparticles which sheathed by several graphene sheets in MWCNTs (Pumera, 2007) may also promote the catalysis of AA, DA and NO_2^-.

3.3. Simultaneous voltammetric determination of AA, DA and NO_2^-

The pH value of the support buffer solution could affect the response of the modified electrode. Fig. 1S depicts the CV behaviors of the modified electrode in DA and AA co-existing various pH value PBS. As can be seen, with the increasing of pH value, the oxidation potentials for DA and AA became more negative. The maximum peak separation between AA and DA was obtained at pH 6.0. Therefore, 0.1 M pH 6.0 PBS was chosen as the supporting buffer solution in the experiment.

The main aim of the present investigation is to determine AA, DA and NO_2^- simultaneous. Fig. 2 reveals the CV behaviors of the biosensor at electrochemical oxidation of AA, DA and NO_2^- mixture. With the adding of AA, DA and NO_2^- mixture, three clear and well-separated peaks were observed, and the peak currents increased linearly on increasing the concentration of AA, DA and NO_2^-. In order to improve the sensitivity as well as determine the linear ranges, different pulse voltammetry (DPV) were employed to record the anodic peak currents (Fig. 2S). Under optimal conditions, AA, DA and NO_2^- could be determined in the ranges of 5 μM–0.48 mM, 0.15–21.65 μM and 5 μM–6.75 mM. The

detection limits (S/N = 3) were 1.65 μM, 0.05 μM and 1.65 μM, respectively. The linear regression equations for AA, DA and NO_2^- were expressed as I_{AA} (μM) = 0.04 C_{AA} (mM) + 41.44, R^2 = 0.992, I_{DA} (μM) = 0.04 C_{DA} (μM) + 5.69, R^2 = 0.997, and $I_{NO_2^-}$ (μM) = 12.68 $C_{NO_2^-}$ (mM) + 0.037, R^2 = 0.985, respectively. The mechanisms behind the oxidation of AA, DA and NO_2^- at a wide potential separation are concluded in the following aspects. First, due to the host–guest chemical reaction, the oxidation of DA in the surface of the electrode is enhanced by its diffusion through the cavities of β-CD and the easy contact with the dispersed GS–MWCNTs that facilitate the electron transfer (Alarcon-Angeles et al., 2008). Second, MWCNTs effectively inhibit the stacking of individual GS and enhance the utilization of GS based composites. Moreover, the surface of the electrode becomes more porous after adding MWCNTs. According to Compton's suggestion that the conducting porous layers on the surface of electrodes can change the mass transport regime from linear (planar) diffusion to one of approximately 'thin layer' character and that this alternation can facilitate the discrimination of many species which oxidize or reduce at similar potentials (Henstridge et al., 2010). Third, the π–π interaction between phenyl structure of DA and GS–MWCNTs makes the electron transfer more feasible than that between penta-heterocycle of AA and GS–MWCNTs (Wang et al., 2009).

3.4. Interference and stability

As demonstrated above, the modified electrode holding intrinsic properties could substantially differentiate AA, DA and NO_2^-. Therefore, the interference from each other can be neglected. Other influences from common co-existing substances in real samples were investigated. When 100-fold concentration of NaCl, $CaCl_2$, citric acid, $MgSO_4$, NaH_2PO_4 and 20-fold glucose, L-tryptophane, L-cysteine were added into 0.1 M pH 6.0 PBS containing 50 μM AA, 5 μM DA and 50 μM NO_2^-, no obvious interference was observed. The stability of the biosensor was also tested. The peak current intensity remained 93.8%, 91.2% and 94.4% for AA, DA and NO_2^-, respectively, after its storage in PBS for a week. The relative standard deviation (RSD) (n = 5) for all these species is less than 3.7%. The results showed that the modified electrodes have high selectivity, well stability and good reproducibility.

3.5. Real samples analysis

Human urine sample was selected as real sample and diluted 1000 times with 0.1 M pH 6.0 PBS before the measurement to fit the calibration curve and reduce the matrix effect. No other pretreatment process was performed. The recoveries ranged between 95.0% and 106.6% (Table 1S), implying that the modified electrode could be efficiently used to simultaneous determination of AA, DA and NO_2^- in real samples.

4. Conclusions

In summary, a novel biosensor was successfully constructed to simultaneous determine DA, AA and NO_2^- based on GS, polycyclodextrin (CDP) and MWCNTs nanocomposites modified GCE. It was found that with the addition of the three molecules, three clear and well-separated peaks were observed, which is ascribed to the host–guest chemical reaction ability of CD and π–π stacking interaction between detected molecules and GS–MWCNTs surface. Additionally, comparing with the behaviors of different modified electrodes, we can conclude that MWCNTs can effectively inhibit the stacking of individual GS and enhance the utilization of GS based composites, which will improved the electrochemical signal of the modified electrode. Moreover, the biosensor showed high selectivity, excellent sensitivity and well stability. Also, such a novel hybrid provides an efficient and promising platform for the detection of other bioelectrochemical devices.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.03.017.

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