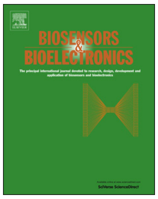
Biosensors and Bioelectronics 57 (2014) 232–238

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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



Overoxidized polyimidazole/graphene oxide copolymer modified electrode for the simultaneous determination of ascorbic acid, dopamine, uric acid, guanine and adenine

Xiaofang Liu, Ling Zhang, Shaping Wei*, Shihong Chen*, Xin Ou, Qiyi Lu

Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 12 January 2014
Received in revised form
6 February 2014
Accepted 9 February 2014
Available online 20 February 2014

Keywords:
Polyimidazole
Graphene oxide
Simultaneous determination
Small biomolecules

ABSTRACT

In the present work, a novel strategy based on overoxidized polyimidazole (PImox) and graphene oxide (GO) copolymer modified electrode was proposed for the simultaneous determination of ascorbic acid (AA), dopamine (DA), uric acid (UA), guanine (G) and adenine (A). The copolymer was characterized by the scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier transform infrared (FT-IR), X-ray photoelectron spectroscopy (XPS) and electrochemical impedance spectroscopy (EIS). Due to the synergistic effects between PImox and GO, the proposed electrode exhibited excellent electrochemical catalytic activities and high selectivity and sensitivity toward the oxidation of AA, DA, UA, G and A. The peak separations between AA and DA, AA and UA, UA and G, and G and A were 140 mV, 200 mV, 380 mV and 300 mV, respectively. The linear response ranges for AA, DA, UA, G and A were 75–2275 µM, 12–278 µM, 3.6–249.6 µM, 3.3–103.3 µM and 9.6–215 µM, respectively, and corresponding detection limits were 18 µM, 0.63 µM, 0.59 µM, 0.48 µM and 1.28 µM.

1. Introduction

Ascorbic acid (AA) is popularly known for its antioxidant properties and presents in the human diet as a vital vitamin. It has been used for the prevention and treatment of scurvy, common cold, mental illness, cancer and AIDS (Noroozifar and Motlagh, 2003). Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system (Huang et al., 2008). Abnormal levels of DA will lead to neurological disorders such as Parkinsonism and schizophrenia (Wightman et al., 1988; Martin, 1998). Uric acid (UA) is the primary end product of purine metabolism. The extreme abnormalities of UA levels will lead to some diseases, such as gout and hyperuricaemia (Dutt and Mottola, 1974). Adenine (A) and guanine (G) are the main nucleotides in deoxyribonucleic acid (DNA). The abnormal changes of A and G in organism suggest the deficiency and mutation of the immunity system and may indicate the presence of various diseases (Shahrokhian et al., 2012). AA, DA, UA, A and G usually coexist in physiological fluids such as serum and aurine (Niu et al., 2013; Balamurugan et al., 2013). Therefore, a sensitive and selective method for simultaneous determination of them is highly desirable for biomedical chemistry and diagnostic research. Unfortunately, previous reports only focus on individual or simultaneous determination of two to four compounds. For example, Huang et al. (2014) utilized Au@carbon dots–chitosan composite as an electrode modifier to detect dopamine. Kul et al. (2013) used poly(Nile blue A) and functionalized multi-walled carbon nanotube modified electrodes to detect ascorbic acid. Sheng et al. (2012) reported the nitrogen doped graphene modified electrode for simultaneous determination of ascorbic acid, dopamine, and uric acid. Yin et al. (2010) utilized the graphene–Nafion composite film modified electrode to simultaneously determine guanine and adenine. Recently, Niu et al. (2013) used the nano-Au/DNA/nano-Au/poly(SFR) composite modified electrode for simultaneous determination of dopamine, uric acid, guanine, and adenine. To the best of our knowledge, no report is available in the literature for simultaneous determination of AA, DA, UA, G, and A.

In the past few decades, electrochemical techniques have received considerable interest for the detection of small biomolecules owing to their high sensitivity, rapid response, and low expense (Zhou et al., 2013; Huang et al., 2011). However, it is very difficult to simultaneously determine five species directly at bare electrodes because the overlapping of their oxidation potentials and the pronounced electrode fouling often result in poor selectivity and reproducibility at bare electrodes (Premkumar and Khoo, 2005; Kumar et al., 2005). To overcome this problem, various materials

---

* Corresponding authors. Tel./fax: +86 23 68253172.
E-mail addresses: shapingw@swu.edu.cn (S. Wei), cshong@swu.edu.cn (S. Chen).

http://dx.doi.org/10.1016/j.bios.2014.02.017
0956-5663

have been utilized to construct and modify the electrodes, such as polymers (Balamurugan and Chen, 2007; Zheng et al., 2008), self-assembled monolayers (Kalimuthu and John, 2006; Shervedani et al., 2006), and nanomaterials including carbon nanotubes (Cui et al., 2012; Abbaspour and Noori, 2011), nanoparticles (Thiagarajan and Chen, 2007; Zhang and Jiang, 2005), and metal oxide (Shakkthivel and Chen, 2007; Li et al., 2000). Specifically, graphene oxide (GO) has attracted considerable attention because it not only owns some properties of graphene with large surface area, thermal, mechanical properties, and capability of chemical modification, but also possesses some other properties such as hydrophilicity, multiple oxygen moieties, and controllable electronic properties (Zeng et al., 2013). Meanwhile, some investigations have been conducted for the application of conducting polymers in the area of sensors (Nie et al., 2013; Kamyabi et al., 2013; Zhuang et al., 2011; Qian et al., 2013). Among variety of conducting polymers, polyimidazole (PIm) is a technologically important material owing to its unique electrical, electrochemical, and optical properties. Imidazole (Im) can be polymerized on the surface of electrode to form chemically stable homogeneous PIm film with controlled thickness (Wang et al., 2012). Furthermore, this PIm film can be overoxidized at high anodic potentials to produce overoxidized polyimidazole (PImox) film. During the overoxidation process, oxygen containing groups such as carbonyl and carboxyl were introduced to the imidazole unit, thus resulting in the improvement of the permselective and antifouling properties of the sensor.

In this paper, the composites of GO and PImox were prepared to construct a sensor for simultaneous determination of AA, DA, UA, G, and A. The obtained PImox–GO copolymer exhibited excellent catalytic activity to AA, DA, UA, G, and A. The electrochemical behaviors of the sensor were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

2. Experimental

2.1. Reagents and materials

Graphene oxide (GO) was obtained from Nanjing Xianfeng Nano Co. (Nanjing, China). Imidazole (Im) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Sodium dodecylsulfate (SDS), uric acid, ascorbic acid, dopamine, adenine, and guanine were purchased from Aladdin in Chemical Reagents Co. Ltd. (Chengdu, China). Phosphate-buffered saline (PBS) solutions (0.10 M) at various pH values were prepared using 0.10 M Na_2HPO_4 and 0.10 M KH_2PO_4. The supporting electrolyte was 0.10 M KCl.

2.2. Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) measurements were performed using a CHI 660A electrochemical workstation (Shanghai Chenhua Instrument, Co., China). Scanning electron microscopy (SEM) was carried out using a Hitachi scanning electron microscope (SEM, S-4800, Hitachi, Japan). Transmission electron microscopy (TEM) was conducted using a TECNAI 10 (PHILIPS FEI Co., Holland). Atomic force microscopy (AFM) images were taken using a scanning probe microscope (Vecco, USA). X-ray photoelectron spectroscopy (XPS) measurements were carried out with a VG Scientific ESCALAB250 spectrometer, using Al KR X-ray (1486.6 eV) as the light source. Fourier transform infrared spectroscopy (FT-IR) measurement was carried out using a Jasco FT/IR-300E instrument (Jasco Ltd., Tokyo, Japan). The conventional three-electrode system included a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and the modified electrode as the working electrode. All measurements were carried out at room temperature.

2.3. Preparation of the sensor

A glassy carbon electrode (GCE, diameter 4.0 mm) was carefully polished with 0.3 and 0.05 μm alumina slurry, and then washed thoroughly with ethanol and ultrapure water. 0.30 mol imidazole monomer was dispersed into 3.0 mL GO solution (5.0 mg mL^{-1}) containing 0.30 mol SDS under ultrasonication for 60 min at room temperature. The PIm–GO composite film was fabricated on the electrode surface by cyclic voltammetry (CV) scanning of GCE in the above mixture solution from -0.20 to 0.80 V with a scan rate of 0.10 V s^{-1} for 8 cycles. Subsequently, the PIm–GO modified electrode was washed with double distilled water and then transferred into 0.10 M PBS (pH 3.0) for electrochemical oxidation at $+1.8$ V for 250 s. The electrode was carefully rinsed with double-distilled water and dried in air as PImox–GO/GCE. For comparison, PImox/GCE and GO/GCE were prepared using the similar procedure.

3. Results and discussion

3.1. Characterization of the PImox–GO

TEM and SEM were employed to investigate the morphologies of modified films. The TEM and SEM images of GO are shown in Fig. 1A and B, respectively. As presented in Fig. 1A and B, the typically crumpled and wrinkled structure of GO was observed. Furthermore, the monolayer structure also could be clearly seen. Fig. 1C shows the SEM image of PImox film without GO, which presented a porous structure. Fig. 1D shows the SEM image of nanocomposite film of PImox–GO. As expected, the GO nanosheets were covered with a porous PImox film, indicating that the PImox–GO nanocomposite film was successfully fabricated on the electrode surface by CV scanning and electrochemical oxidation process.

AFM was conducted to observe the surface topography of PImox–GO nanocomposite and a typical topographic AFM image is shown in Fig. 1E. From Fig. 1E, a porous layer of composite film was clearly observed, which was due to the electrodeposition of imidazole monomer on the indium tin oxide (ITO) surface. Herein, GO could not be clearly observed in the AFM image of PImox–GO. It was probable that the GO nanosheets were covered with a uniform nanoparticle layer of PImox.

FT-IR spectra were employed to characterize the formation of the PIm–GO copolymer. As seen from Fig. 2A (curve a), the FT-IR spectrum of PIm exhibited C=C band of aromatic ring at 1642 cm^{-1} (Tian et al., 2013) and C=N– stretching band at 1468 cm^{-1} (Raj et al., 2012). In addition, two peaks at 2850 and 2918 cm^{-1} were due to C–H stretching vibrations. Fig. 2A (curve b) shows the FT-IR spectrum of PIm–GO nanocomposite. Compared with the FT-IR spectrum of PIm (curve a), the peak of C=C obviously became stronger in the FT-IR spectrum of PIm–GO (curve b). Furthermore, two new peaks appeared at 1204 cm^{-1} and 3435 cm^{-1} (curve b), which were ascribed to C–O band and O–H band of GO, respectively (Yang et al., 2014). This fact demonstrated that PIm–GO nanocomposite was successfully fabricated.

To gain the information concerning the chemical composition in PImox–GO nanocomposite, XPS measurements were performed on the PIm–GO and PImox–GO modified films and the results are shown in Fig. 2B. As seen from Fig. 2B, a N 1s peak was observed at the XPS spectra of both PIm–GO (curve a) and PImox–GO (curve b), which was attributed to imidazole. In addition, both PIm–GO