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Simultaneous electrochemical determination of guanosine and adenosine with graphene–ZrO_2 nanocomposite modified carbon ionic liquid electrode

Wei Sun [a,b,*], Xiuzhen Wang [b], Xiaohuan Sun [b], Ying Deng [b], Jun Liu [b], Bingxin Lei [a], Zhenfan Sun [a]

[a] Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou 571158, PR China
[b] College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, PR China

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ABSTRACT

In this paper an ionic liquid 1-hexylpyridinium hexafluorophosphate based carbon ionic liquid electrode (CILE) was fabricated and used as the basal electrode, which was further modified by graphene (GR) and ZrO_2 nanoparticle with chitosan (CTS) film to immobilize the nanocomposite. The modified electrode was denoted as CTS–GR–ZrO_2/CILE and further used for the simultaneous detection of adenosine and guanosine. Electrochemical performances of the modified electrode were greatly enhanced due to the presence of GR–ZrO_2 nanocomposite, and the direct electro-oxidation behaviors of adenosine and guanosine were carefully investigated. Both adenosine and guanosine exhibited an increase of the oxidation peak currents with the negative shift of the oxidation peak potentials on the modified electrode, which indicated the electrocatalytic activity of GR–ZrO_2 nanocomposite on the electrode surface. Electrochemical parameters of adenosine and guanosine on CTS–GR–ZrO_2/CILE were calculated respectively, and a new electroanalytical method for the simultaneous determination of adenosine and guanosine was further established with the peak-to-peak separation (ΔEp) as 0.225 V. The proposed method was successfully applied to detect adenosine and guanosine in human urine samples with satisfactory results.

1. Introduction

As a two-dimensional (2D) carbon material which is comprised of sp^2 hybridized single sheet carbon atoms, graphene (GR) has been widely investigated due to its unique physical and chemical properties, such as good thermal conductivity, high charge carrier mobility at room temperature and big specific surface area (Choi et al., 2010). Due to its specific electrochemical prosperities, GR and related materials have been used in different fields of electrochemistry including supercapacitor, Li-ion batteries, electrocatalysis and electrochemical sensors (Chen et al., 2010; Ratinac et al., 2011; Brownson and Banks, 2010). Hou et al. (2011) summarized the recent research and development of GR based electrochemical energy conversion and storage system. Liu et al. (2012) reviewed the emerging GR based sensors for the biological and chemical detection. Brownson et al. (2012) overviewed the fundamental concepts of GR electrochemistry and its prominent applications. Shan et al. (2010) applied ionic liquid (IL)-functionalized GR to construct an electrochemical biosensor for detection of NADH. Wu et al. (2010) proposed a chitosan-dispersed GR modified glassy carbon electrode (GCE) for the direct electron transfer of cytochrome *C*. Our group also applied GR composites modified electrode for the investigation on the protein electrochemistry (Ruan et al., 2012) and the detection of hydroquinone (Hu et al., 2012) or bisphenol A (Wang et al., 2012). So GR modified electrode has been devised for the electrochemical application. But GR nanosheets tend to aggregate back to graphite on the electrode surface, which limit its real application. In recent years GR based nanocomposites have been studied, which can modify the GR nanosheets and avoid the agglomeration. The presence of inorganic particles on the GR surface can not only prevent the restacking but also form a class of GR based nanocomposite with many new functions (Singh et al., 2011). Bai and Shen (2012) presented a review about the synthesis and application of GR–inorganic nanocomposites. ZrO_2 nanoparticle is a commonly used inorganic oxide with good thermal stability, chemical inertness and lack of toxicity (Dobson and McQuillan,

* Corresponding author at: Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou 571158, PR China. Tel./fax: +86 898 31381637.
E-mail address: swyy26@hotmail.com (W. Sun).

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1997) and affinity for the groups containing oxygen (Fang et al., 1997), which has been used in the modified electrodes. Liu et al. (2004) investigated the direct electron transfer of hemoglobin on ZrO_2 nanoparticle modified pyrolytic graphite electrode. Du et al. (2008) applied a ZrO_2 nanoparticle modified electrode for the stripping voltammetric analysis of organophosphate pesticides. Zhu et al. (2004) fabricated an electrodeposited ZrO_2 thin films modified gold electrode for electrochemical detection of DNA hybridization. Recently GR–ZrO_2 nanocomposite had been synthesized by different methods. Yin et al. (2012) fabricated a GR–ZrO_2 nanocomposite by simple mechanical mixing and pressureless sintering process, which resulted in a homogeneous and random distribution of GR in the ZrO_2 matrix. Du et al. (2011) described a one-step electrodeposition method for GR–ZrO_2 nanocomposite modified GCE and used for the detection of organophosphorus agents. Gong et al. (2012) also used a similar electrodeposition method for the synthesis of ZrO_2 nanoparticles decorated GR hybrid nanosheets. The combination of GR–ZrO_2 nanocomposite exhibits the individual characteristics of GR such as large surface area and good conductivity with that of ZrO_2 nanoparticles such as biocompatibility and enrichment ability of oxygen groups, which has great potential applications in the field of electrochemical sensors.

Purine nucleotides have exhibited many important metabolic and biological effects in human systems, and the concentration changes of these nucleosides in body fluids can be used to indicate various pathological changes such as carcinoma or liver disease (Yang et al., 2002), so it is necessary to establish sensitive methods for the nucleotides detection. Guanosine and adenosine are two important nucleosides that present in the molecular structure of nucleic acids, which are vital in various biological processes. For example, adenosine can modulate physiological functions in heart and brain, and regulate oxygen supply during cell stress and renal function (Kloor et al., 2000). Guanosine plays a protective role during brain ischemia (Frizzo et al., 2002) and mediates the process of RNA splicing (Piriev et al., 1998). Many analytical methods have been developed for the individual or simultaneous determination of guanosine and adenosine. Lin et al. (1997) achieved the simultaneous determination of ribonucleosides by capillary electrophoresis with a copper working electrode. Giannattasio et al. (2003) achieved simultaneous determination of purine nucleotides by ion-pair high performance liquid chromatography. Chen et al. (2008) reported an Ag–clad Au colloids film for the detection of adenosine using surface enhanced Raman scattering sensing platform based on a structure switching aptamer. Goyal et al. (2007) used a fullerene-C_{60} modified GCE for the voltammetric detection of adenosine and guanosine.

In this work a GR–ZrO_2 nanocomposite was fabricated and further modified on a 1-hexylpyridinium hexafluorophosphate ($HPPF_6$) based carbon ionic liquid electrode (CILE). The modified electrode was further used for the detection of guanosine and adenosine. IL is a kind of green solvent with the characteristics such as high ionic conductivity, wide electrochemical windows and good solubility. Due to its specific properties IL had been used as the electrolyte or the modifier in the field of electrochemical sensor (Wei and Ivaska, 2008). CILE is a new type of working electrode that is prepared by using IL as the binder and the modifier in the traditional carbon paste electrode (CPE). Due to the presence of IL in the carbon paste, CILE had shown the advantages such as increased conductivity, easy preparation, good reversibility, high sensitivity and the ability to lower the overpotential of electroactive compounds (Maleki et al., 2006). Our group applied an N-butylpyridinium hexafluorophosphate modified CPE for the investigation on the electrochemical behaviors of guansoine (Sun et al., 2009), and simultaneously detection of adenosine and guanosine on 1-ethyl-3-methylimidazolium ethylsulfate based CILE (Sun et al., 2011). The results showed that the modifier could promote the electrochemical performance with the simultaneous determination of guanosine and adenosine realized on the modified electrode. Due to the advantages of GR and ZrO_2 nanoparticles and their synergistic effects, the GR–ZrO_2 nanocomposite modified electrode was prepared and exhibited better electrochemical performances. Based on the electrochemical response of guanosine and adenosine on CTS–GR–ZrO_2/CILE, a new electrochemical method was established for the simultaneous determination with good electrocatalytic activity, high sensitivity, good repeatability, long-term stability and low cost.

2. Experimental

2.1. Apparatus

Cyclic voltammetry and differential pulse voltammetry were carried out on a CHI 1210A electrochemical workstation (Shanghai CH Instruments, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 750B electrochemical workstation (Shanghai CH Instrument, China). A conventional three-electrode system was used with a CTS–GR–ZrO_2/CILE (Φ=4 mm) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. Scanning electron microscopy was performed on a JSM-6700F scanning electron microscope (Japan Electron Company, Japan).

2.2. Reagents

Guanosine (99%, Sigma), adenosine (99%, Sigma), 1-hexylpyridinium hexafluorophosphate ($HPPF_6$, Lanzhou Greenchem ILS. LICP. CAS., China) and graphite powder (average particle size of 30 μm, Shanghai Colloid Chemical Co., China) were used as received. Graphene oxide (GO) was synthesized according to the previous reports (Hummers and Offerman, 1958), which was further reduced to GR with the addition of hydrazine (Wang et al., 2009). The final blank GR were obtained by filtration and dried in vacuum. ZrO_2 nanoparticle was prepared according to a reported method (Kim et al., 2009). 0.2 mol L^{-1} Britton–Robinson (B–R) buffer solutions with various pH values were used as the supporting electrolyte. Urine samples received from healthy laboratory personnel were used in the electrochemical measurement after 50 times dilution with B–R buffer solution. All the other chemicals were of analytical reagent grade and doubly distilled water was used in all the experiments.

2.3. Preparation of CTS–GR–ZrO_2/CILE

CILE was fabricated by mixing 0.8 g of $HPPF_6$ and 1.6 g of graphite powder in a mortar and ground carefully. A portion of resulted homogeneous paste was packed firmly into a glass tube cavity (Φ=4 mm) and the electrical contact was established through a copper wire to the end of the paste in the inner hole of the tube. The surface of CILE was polished on a piece of polishing paper just before used. 0.5 mg of GR and 0.8 mg of ZrO_2 nanoparticle were dispersed into 1.0 mL of 1.0% CTS solution (in 1.0% HAc) and ultrasonicated for 2 h to form a homogenous solution. Then 7.0 μL of CTS–GR–ZrO_2 mixture was dropped on CILE surface and dried in the air. After the solvent was evaporated, the electrode was thoroughly rinsed with water and the modified electrode was noted as CTS–GR–ZrO_2/CILE. For comparison CTS–ZrO_2/CILE and CTS–GR/CILE were also fabricated with the similar procedure.