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Simultaneous electroanalysis of isoniazid and uric acid at poly(sulfosalicylic acid)/electroreduced carboxylated graphene modified glassy carbon electrode

Hongling Yan[a], Hualing Xiao[a], Qingji Xie[a,*], Jiali Liu[a], Lingen Sun[a], Yaping Zhou[a], Yi Zhang[a], Long Chao[a], Chao Chen[a,*], Shuozhuo Yao[a,b]

[a] Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, China
[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China

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ABSTRACT

Simultaneous electroanalysis of isoniazid and uric acid (UA) was achieved at a poly(sulfosalicylic acid) (PSA) and carboxylated graphene (CG)-modified glassy carbon electrode (GCE). The CG cast-coated on the GCE was cathodically reduced to form electroreduced CG (ERCG), while the PSA was electrochemically synthesized on the ERCG/GCE. Electrochemical quartz crystal microbalance was used to investigate the electrodeposition processes. The surface morphologies and electrochemical properties of the PSA/ERCG/GCE were studied by scanning electron microscopy, cyclic voltammetry (CV) and electrochemical impedance spectroscopy. The electrochemical behaviors of isoniazid and UA at PSA/ERCG/GCE were investigated by CV and differential pulse voltammetry (DPV), giving well defined and well separated oxidation peaks of isoniazid and UA. The PSA/ERCG/GCE exhibited notably enhanced electro-oxidation signals of isoniazid and UA in NH_3-NH_4Cl buffer solution (pH 9.0), as compared with bare GCE and PSA/GCE. Under optimum conditions, the DPV peak currents at PSA/ERCG/GCE responded linearly to isoniazid concentration from 0.05 to 15 μM and to UA concentration from 0.02 to 15 μM, with limits of detection of 12 nM for both isoniazid and UA, which is an improvement compared to many other reported techniques. The PSA/ERCG/GCE was successfully applied to the simultaneous determination of isoniazid and UA in urine substrate samples.

1. Introduction

Isoniazid (pyridine-4-carboxylic hydrazide) is an important antituberculosis drug in the clinical practice for chemotherapy of tuberculosis. However, isoniazid can cause hepatotoxicity in patients with an inflammatory response and even death following long-term therapy of isoniazid [1,2]. Therefore, the assay of isoniazid level in human body fluids is vital for the effective therapeutic dosages, which makes it necessary to develop rapid and effective methods for the quantitative analysis of isoniazid in human body fluids. Many analytical methods have been developed for the determination of isoniazid, including high-performance liquid chromatography [3,4], gas chromatography [5], capillary electrophoresis [6], chemiluminescence [7,8], ultraviolet-visible spectrophotometry [9,10], fluorimetry [11] and electroanalytical methods [12,13]. Among these methods, electroanalytical methods have been reported for quantifying the drug concentration in blood or urine with high sensitivity, simplicity and reproducibility [14–16]. For electroanalysis of isoniazid, the high overpotential required for the oxidation of isoniazid at an electrode (e.g., >0.8 V at a carbon nanotube based electrode [17]) is a major concern. Therefore, many methods have been developed to decrease the overpotential of isoniazid using chemically modified electrodes to catalyze the oxidation of isoniazid. Various types of electrodes have been developed to determine isoniazid, including gold electrode [18], dropping mercury electrode [19], overoxidized polypyrrole modified glassy carbon electrode (GCE) [12], poly(amidosulfonic acid) modified GCE [20], ordered mesoporous carbon modified GCE [21], electrochemically reduced

* Corresponding authors. Tel.: +86 731 88865515; fax: +86 731 88865515.
E-mail addresses: xieqj@hunnu.edu.cn (Q. Xie), chenchao840103@163.com (C. Chen).

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graphene oxide modified GCE [22], thionine immobilized multiwalled carbon nanotube modified carbon paste electrode [23], and poly(L-histidine) modified screen-printed carbon electrode [13]. In our opinion, there is still a need for developing high-performance new electrodes for rapid assay of isoniazid in real samples.

Uric acid (2,6,8-trihydroxypurine, UA) is the primary end product of purine metabolism in the human body. The normal uric acid level in urine is between 1.49 and 4.46 mM [24]. Abnormal levels of UA (e.g., >4.46 mM) are symptoms of several diseases, such as gout and hyperuricemia [25,26]. Many epidemiological studies have also suggested that serum UA is a risk factor for cardiovascular disease [27,28]. Therefore, the determination of UA with simple and effective methods is of clinical significance. For this aim, various methods have been used, such as spectrofluorometry [29], chromatography [30,31], enzymatic methods [32,33], chemiluminescence [34], and electroanalytical methods [35,36] and so forth. The electroanalysis based on the direct electrochemistry of UA is promising for rapid analysis of UA. Given that the oxidation peak potentials of isoniazid and UA are close to 0.58 V and 0.47 V (versus KCl-saturated calomel electrode (SCE)) [37,38], respectively, there is an overlap in the two oxidation signals. This problem becomes more severe in detecting isoniazid in physiological samples (e.g. urine) that contain UA at high concentrations. Therefore, we must consider the impact of UA during electroanalysis of isoniazid, and it is also necessary to develop simple and effective methods for the simultaneous analysis of isoniazid and UA in body fluids.

Graphene is a single layer of carbon atoms packed into two-dimensional honeycomb lattice. Because of the unique properties, such as high electrical conductivity, exceptional thermal and mechanical properties as well as high specific surface area, graphene and its composites have attracted worldwide interest for potential applications in fabricating electrochemical sensors [39]. However, graphene is hydrophobic and tends to agglomerate, which limits its applications in many cases. Great efforts have been made to synthesis of water-dispersable graphene material via chemical or physical functionalization, such as anionic carboxylated graphene (CG) [40] and anionic sulfonated graphene [41]. The anionic graphene derivatives with enhanced water-dispersability are also expected to behave as a cation-permselective film for more selective electroanalysis of cationic isoniazid. We can electropolymerize functional molecules on the surfaces of graphene and its derivatives for improved application performance, for example, electropolymerization of anionic sulfosalicylic acid (SA) on CG to enhance the cationic permselectivity of the CG film. Poly(sulfosalicylic acid) (PSA) modified electrode has been reported as an electrochemical sensor to determine biomolecules [42,43]. However, the GCE modified with PSA and CG has not been used for the simultaneous electroanalysis of isoniazid and UA to date.

Herein, we have fabricated a PSA/electroreduced CG (ERCG) modified GCE (PSA/ERCG/GCE) for the simultaneous analysis of isoniazid and UA. The motivation of this work is as follows. Partial electroreduction of CG gives highly electron-conductive ERCG of large specific surface area with some anionic carbonyl groups (COO^-) remaining, and PSA contains many anionic sulfonic acid groups (SO_3^-), thus the PSA/ERCG film can be negatively charged at high pH. At pH 9.0, isoniazid (pK_a = 10.8 [44]) is cationic, but UA is anionic (pK_a = 3.1 [45]). Therefore, isoniazid and UA can show sufficiently different electrochemical behaviors on the PSA/ERCG/GCE. As expected, the PSA/ERCG/GCE showed excellent selectivity (well-separated anodic peaks of isoniazid and UA) and high sensitivity for the simultaneous electroanalysis of isoniazid and UA in real samples.

2. Experimental

2.1. Apparatus and chemicals

Electrochemical experiments were conducted with a CHI660A electrochemical workstation (CH Instruments, USA) and a three-electrode system that consisted of a disk GCE (3 mm diameter) as the working electrode, a KCl-saturated calomel electrode (SCE) as the reference electrode, and a carbon rod as the counter electrode. All potentials here are referenced to SCE. A computer-interfaced HP4395A impedance analyzer (Agilent, USA) was employed in the quartz crystal microbalance (QCM) and electrochemical QCM (EQCM) experiments. AT-cut 9-MHz gold-coated piezoelectric quartz crystals (PQCs, 12.5 mm wafer-diameter and 6.0 mm electrode-diameter, Model JA5, Beijing Chenjing Electronics Co. Ltd., China) were used. The AT-cut crystals are cut at an angle of approximately 35° with respect to the optical axis, which exhibit high frequency stability and possess a very low frequency–temperature coefficient between 0 and 50 °C in air. The PQC was sealed at the end of a cut Eppendorf tube using silicon rubber adhesive, so that one face of the PQC was in contact with the solution and the other face of the PQC was in air. Scanning electron microscopy (SEM) pictures were collected on a JEM-6700F field emission scanning electron microscope. Fourier transform infrared (FT-IR) spectra were collected on a Nicolet Nexus 670 FT-IR instrument (Nicolet Instrument Co., USA) in its transmission mode. The pH of buffers was measured with a pH meter (Leici PHS-3C, Shanghai Precision & Scientific Instrument Co. Ltd., China).

CG (purity > 99%) and graphene oxide (GO) were purchased from Nanjing XFNANO Materials Tech Co., Ltd. (China). Isoniazid, UA and ascorbic acid (AA) were obtained from Sigma. SA was purchased from DaMao Chemical Reagent (Tianjin, China). Anhydrous ethanol (99.5%) was purchased from the Second Reagent Factory of Shanghai. 0.1 M phosphate buffer solutions (PBS, 0.019 M NaH_2PO_4 + 0.081 M Na_2HPO_4 + 0.1 M Na_2SO_4 at pH 7.4, and 0.094 M NaH_2PO_4 + 0.006 M Na_2HPO_4 at pH 5.5) were prepared. Britton–Robinson (BR) buffer (0.04 M boric acid + 0.04 M phosphoric acid + 0.04 M acetic acid) was prepared and then 0.2 M NaOH was used to adjust its pH to the desired value, as monitored by a pH meter. NH_3-NH_4Cl buffer was prepared with $NH_3{\cdot}H_2O$ and NH_4Cl, the pH of which was adjusted by changing the molar ratio of $NH_3{\cdot}H_2O$ to NH_4Cl. Other chemicals used were of analytical grade or better quality and Milli-Q ultrapure water (>18 MΩ cm) was used throughout the experiments.

2.2. Procedures

Prior to use, the GCE was sequentially polished with 1.0 and 0.05 μm alumina slurry to obtain a mirror-like surface, then washed ultrasonically in water and ethanol for 5 min, respectively. Then, the GCE was subjected to potential cycling (−0.2 ~ 1.0 V, 100 mV s^{-1}) in 0.20 mol L^{-1} aqueous $HClO_4$ until reproducible cyclic voltammograms were obtained. The cleaned GCE was cast-coated with 4 μL 0.25 mg mL^{-1} CG solution and dried in air. After washing with water, the CG modified on the GCE was electroreduced in 0.1 M PBS (pH 7.4) by cyclic voltammetry (CV) between −1.3 and 0 V at 50 mV s^{-1} for 10 cycles. Then, the ERCG/GCE was washed with water and immersed in 0.1 M PBS (pH 5.5) containing 5 mM SA to conduct the CV polymerization of SA (−1.0 ~ 2.0 V, 100 mV s^{-1}, 10 cycles). Finally, the electrode was washed with water to remove any non-adsorbed materials and the PSA/ERCG/GCE was obtained. For comparison, ERCG/GCE, electroreduced graphene oxide (ERGO)/GCE and PSA/GCE were similarly prepared in the