Sensors and Actuators B 150 (2010) 43–49

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Electrochemical sensor for simultaneous determination of uric acid, xanthine and hypoxanthine based on poly (bromocresol purple) modified glassy carbon electrode

Yan Wang*, Li-li Tong

College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan, Shandong 250014, China

ARTICLE INFO

Article history:
Received 26 April 2010
Received in revised form 8 July 2010
Accepted 27 July 2010
Available online 3 August 2010

Keywords:
Bromocresol purple
Chemical modified electrode
Uric acid
Xanthine
Hypoxanthine

ABSTRACT

A novel electrochemical sensor based on electroactive-polymerized film of bromocresol purple (BCP) modified on glassy carbon electrode for simultaneous determination of uric acid (UA), xanthine (XA) and hypoxanthine (HX) was presented. The preparation and basic electrochemical performance of poly (BCP) film modified glassy carbon electrode were investigated firstly in details. The electrochemical behaviors of UA, XA and HX at the modified electrode were studied by cyclic voltammetry. The results showed that this new electrochemical sensor exhibited excellent electrocatalytic activity towards the oxidation of the three analytes. The anodic peaks of the three species were well defined with lowered oxidation potential and enhanced oxidation peak currents, so the poly (BCP) modified electrode was used for simultaneous voltammetric measurement of UA, XA and HX by differential pulse voltammetry. Under the optimum conditions, the calibration curves for UA, XA and HX were obtained over the range of 0.5–120, 0.1–100 and 0.2–80 μmol L^{-1}, respectively. The detection limits for UA, XA and HX were 0.2, 0.06 and 0.12 μmol L^{-1}, respectively. With good selectivity and sensitivity, the proposed method has been applied to simultaneous determination of UA, XA and HX in human serum with satisfactory results.

1. Introduction

Uric acid (UA), xanthine (XA) and hypoxanthine (HX) are degradation products of purine metabolism in human beings and higher primates. UA is produced by xanthine oxidase from XA and HX, which in turn are produced from purine, so XA and HX are intermediates and UA is the final oxidation product of purine degradation metabolism. The three products can penetrate cell membranes and accumulate in extracellular fluids [1]. The concentration levels of them in body fluids such as human serum and urine are markers of many clinical conditions, including perinatal asphyxia, cerebral ischemia, hyperuricemia and gout, hence accurate detection and quantification of UA, XA, and HX in body fluids are critically important in study of the homeostasis of the xanthine oxidase system and clinical diagnosis at early stages of related diseases [2]. Current methods used to determine simultaneously the purine degradation products are high-performance liquid chromatography (HPLC) [3–5], capillary electrophoresis (CE) [6–8] and electrochemistry [9–16], in which HPLC methods require fastidious sample preparation, prolonged analysis time and expensive material, and CE methods need expensive apparatus.

The development of electrochemical sensors through electrocatalysis for the determination of biologically important compounds is a major interest in current research [17,18]. Various biosensors based on the immobilized enzyme on electrode for determination of the three species has been reported [9,10], but these enzymatic methods are more expensive and lack stability and sensitivity. Non-enzymatic electrochemical sensors, on the other hand, are relatively sensitive, simple, cheap and rapid. It is even possible to determine the three compounds simultaneously. However, only a few reports appeared for the simultaneous determination of UA, XA and HX by non-enzymatic electrochemical approaches [11–16]. Yao et al. first studied the voltammetric oxidation of UA, XA and HX with a glassy carbon electrode, but the method suffered from serious interference by ascorbic acid and from nonlinear dependence of the current response on the concentration of these compounds [11]. Cai et al. proposed a method for the simultaneous differential pulse voltammetric determination of the three compounds with an electrochemically pretreated carbon paste electrode [12]. Zen et al. performed the simultaneous determination of HX, XA and UA using preanodized nontronite coated screen-printed carbon electrode (NSPE) [13], with detection limits of 0.34, 0.07 and 0.42 μmol L^{-1} for them, respectively. Recently, Wang's group developed an inlaying ultra-thin carbon paste electrode modified with functional single-wall carbon nanotubes (SWNTs/IUTCPE) for simultaneous determination of three

* Corresponding author. Tel.: +86 531 89212269; fax: +86 531 88956828.
E-mail address: fagong@sdu.edu.cn (Y. Wang).

0925-4005/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2010.07.044

purine derivatives [14]. Most recently, Kalimuthu and Abraham John described the simultaneous determination of ascorbic acid (AA), dopamine (DA), UA and XA using an ultra-thin electropolymerized film of 2-amino-1,3,4-thiadiazole (p-ATD) modified glassy carbon electrode [15]. Kumar's group reported Ru $(DMSO)_4Cl_2$ nano-aggregated Nafion membrane modified electrode for simultaneous electrochemical detection of HX, XA and UA in human urine [16]. In this paper, we prepared a new non-enzymatic electrochemical sensor based on electropolymerized film of bromocresol purple to catalyze the oxidation of UA, XA and HX, which provided a simple and sensitive voltammetric method for determining the concentrations of the three compounds simultaneously.

Bromocresol purple (BCP), 5′,5″-dibromo-o-cresolsulfophthalein is a pH indicator. It is also used as dye to measure albumin in medical laboratories. Its electropolymerization at the electrode surface and its function as an electrocatalyst have never been reported in the literature. In this work, we reported for the first time on a polymer film of BCP to modify glassy carbon electrode (GCE) and described the electrochemical behaviors of the novel poly (BCP) modified glassy carbon electrode. The poly (BCP) modified electrode possessed the larger real surface area, π–π conjugated bond, a great deal of active sites and better conductivity, which led to the dissimilar conjugation effect of the purine derivatives with the electrode interface. Therefore, the electrochemical reversibility of the oxidation of UA, XA and HX may be greatly improved in the presence of the poly (BCP) film by accelerating the rate of electron transfer, which indicated that the poly (BCP) film had excellent electrocatalytic activity for oxidation reactions of UA, XA and HX at the surface of the modified electrode. Moreover, the modified electrode showed good sensitivity, selectivity and reproducibility for the simultaneous determination of UA, XA and HX. Based on its excellent characteristics compared to other electrochemical sensors reported in terms of high sensitivity, wide linearity and good stability, the poly (BCP) modified electrode was satisfactorily used for the simultaneous determination of UA, XA and HX in human serum by differential pulse voltammetry (DPV).

2. Experimental

2.1. Chemicals

UA, XA and HX were purchased from Sigma (USA). BCP and ascorbic acid were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and were used without further purification. The 0.067 mol L^{-1} phosphate buffer solutions (PBS) with various pH values were prepared by mixing the stock solutions of 0.067 mol L^{-1} KH_2PO_4 and Na_2HPO_4. All solutions were prepared with doubly distilled water.

2.2. Apparatus

Electrochemical measurements were performed with a LK2005 Microcomputer-based electrochemical system (LANLIKE, Tianjin, China). A conventional three-electrode cell was used, including a saturated calomel electrode (SCE) as reference electrode, a platinum sheet electrode as the counter electrode and a bare or modified glassy carbon disk electrode (GCE) with a diameter of 3.0 mm used as working electrode. All pH measurements were made with a pHS-3C digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode. A KQ-250B ultrasonic washer (Kunshan Ultrasonic Instrument Works, Kunshan, China) was used to wash the electrode.

2.3. Preparation of the poly (BCP) modified electrode

Cyclic voltammetry (CV) was used to form polymerization film. Prior to its modification, the bare GCE was polished with 0.05 μm α-alumina powder and rinsed with 1:1 HNO_3 solution, ethanol, and doubly distilled water for 10 min successively. After the electrode was pretreated electrochemically by scanning in a 0.5 mol L^{-1} H_2SO_4 solution between −0.5 and 1.5 V at 100 mV s^{-1} for ten times to get a stable background current, the BCP modified electrode was prepared by electropolymerization. The polymertic film was deposited by cyclic sweeping from −1.0 to 1.5 V at 100 mV s^{-1} for 20 cycles in 0.01 mol L^{-1} $NaNO_3$ solution containing 2.0×10^{-3} mol L^{-1} BCP. After polymerization, the modified electrode was washed with doubly distilled water, and then air-dried.

2.4. Experimental methods

Cyclic voltammetric and differential pulse voltammetric measurements were carried out with three electrodes in phosphate buffer solution. The cyclic voltammograms were recorded by cycling the potential between 0.0 and +1.4 V at a scan rate of 100 mV s^{-1}. The differential pulse voltammetric measurements were performed by applying a sweep potential from 0.0 to +1.2 V at pulse amplitude of 50 mV and pulse width of 0.1 s. All experiments were carried out at room temperature. The poly (BCP) modified electrode could be used repeatedly after rinsed with doubly distilled water and blotted with filter paper.

2.5. Sample preparation

Blood samples were collected from healthy volunteers at the Hospital of Shandong Normal University. A 1.0 mL of fresh blood sample was obtained and centrifuged at 3000 rpm for 20 min to remove all precipitating materials. After the separated serum was diluted 20-fold with pH 6.5 phosphate buffer solution, an aliquot 10.0 mL of this test solution was transferred into the electrochemical cell to detect UA, XA and HX simultaneously by the proposed DPV method.

3. Results and discussion

3.1. Preparation and characterization of electropolymerized BCP film at the GCE surface

Cyclic voltammetry was used to form the electropolymerization film. The potential scan range was the most important factor in preparing poly (BCP) film. If the positive potential value for polymerization was below 1.0 V or if the negative one was above −0.8 V, no polymer reaction occurred. The experimental result showed that the polymeric film formed was more conductive when the potential scan window was from −1.0 to 1.5 V. Therefore, it was selected as the electropolymerization potential window in this paper. Compared with other supporting electrolyte such as phosphate buffer solution used in electrodeposition process of polymertic film, the obtained polymertic film was more complete, uniform and compact, which showed better electrocatalytic activity to the oxidation of UA, XA and HX when $NaNO_3$ was used as supporting electrolyte during polymer formation. Thus $NaNO_3$ was chosen as supporting electrolyte for electropolymerization in this work.

The consecutive cyclic voltammograms of 2.0×10^{-3} mol L^{-1} BCP in 0.01 mol L^{-1} $NaNO_3$ solution at bare glassy carbon electrode were shown in Fig. 1. In the first cycle, one strong anodic peak was observed at 0.800 V(peak A), which might correspond to the oxidation of BCP monomer. A cathodic peak also appeared at about −0.480 V (peak B) in the first cycle. From the second cycle on, a new oxidation peak appeared with potential at 0.520 V (peak C)