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Simultaneous electrochemical sensing of cysteine, uric acid and tyrosine using a novel Au-nanoparticles/poly-Trypan Blue modified glassy carbon electrode

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ABSTRACT

In this study, Au-nanoparticles/poly-Trypan Blue modified glassy carbon electrode (AuNPs/poly-TrB/GCE) was fabricated for the simultaneous determination of cysteine (Cys), uric acid (UA) and tyrosine (Tyr). There are limitations in bare glassy carbon electrode (GCE) in showing clear oxidation peaks for Cys, UA and Tyr; whereas AuNPs/poly-TrB modified electrode completely separated the voltammetric signals of Cys, UA and Tyr at +0.40, +0.58 and +0.90 V, respectively (vs. Ag/AgCl electrode). This modified electrode shows excellent electrocatalytic activity towards the oxidation of Cys with a potential shift about 460 mV to a less positive potential. Under optimized experimental conditions, the detection limits of Cys, UA and Tyr were calculated as 0.006, 0.07 and 0.008 μmol L^{-1}, respectively, at this simple construction sensor. Finally, the fabricated sensor was satisfactorily used for the simultaneous determination of these molecules in human biological samples.

1. Introduction

Free radicals cause many diseases including atherosclerosis, cancer and rheumatoid arthritis. Antioxidants with low molecular weight are among the most important sources that protect human body against free radicals. Urate, which is one of the most potent radical scavengers is the major form of uric acid molecule within the physiological pH range [1]. Among amino acids, cysteine (Cys) and tyrosine (Tyr) are also two major targets for radical attack [2]. Tyrosine is a non-essential amino acid for the production of several important brain chemicals called neurotransmitters including epinephrine, norepinephrine, and dopamine. Tyrosine also supports synthesis melanin, the pigment responsible for hair and skin color. Cys is a semi-essential amino acid with the formula $HO_2CCH(NH_2)CH_2SH$. The thiol side chain in cysteine molecule mostly participates as a nucleophilein enzymatic reactions. Numerous studies indicate that Cys deficiency can result in serious illnesses such as slow growth, hematopoiesis reduction, leucocyte loss, liver damage and Parkinson's disease [1]. Therefore, the development of an impressive method for simultaneous determination of Cys, UA and Tyr has an incredible significance in biochemistry and biomedicine research [3–6]. Many analytical techniques such as spectrophotometric [7,8], chemiluminescence [9,10], fluorometric [11,12] and high performance liquid chromatography with UV detection [13,14] are available for individual determination of Cys, UA and Tyr. However, electrochemical methods with various chemically modified electrodes have been reported in the literature for both individual and Simultaneous determination of these ternary compounds [15–18].

Electropolymerization is a one-step synthesis of a stable polymer film on different substrates. This technique depends on some parameters including applied potential windows, pH of solution and film thickness. The electrically conducting polymers possess unique properties, which can serve as an effective matrix to deposit metal nanoparticles. Trypan Blue (TrB) is an acid azo dye commonly used as a stain to distinguish viable from non-viable cells. It contains two amine groups and phenolic O—H, which can form an electropolymerized film on the surface of glassy carbon electrode. The electropolymerization of az dyes at the surface of electrodes has widely been reported in the literature; for instance, Sulfonazo III, Eriochrome black T, Evans Blue and Adizol black B [19–23]. A new azo bond, which makes an increase in conjugation length and stability has been formed in polymerization process of these compounds. On the other hand, metal nanoparticles (NPs) such as Pt, Au, Cu and PdNPs have exceptionally been applied in biosensor due to their excellent performance [24–26]. Application of conductive

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polymers and metal nanoparticles in biosensor fabrication shows a synergic effect, consequently enhancing the performance of biosensors. This work developed the electropolymerization of TrB and the incorporation of AuNPs to modify the GCE by taking the good performance of AuNPs in electrochemical sensors into consideration. In this study, AuNPs/poly-TrB modified glassy carbon electrode serves as an excellent electrocatalyst property towards the oxidation of Cys, UA and Tyr.

2. Experimental

2.1. Apparatus and reagents

All electrochemical experiments were performed using a Metrohm instrument, Model 797 VA processor. A conventional three–electrode electrochemical system consists of a working electrode (AuNPs/poly-TrB/GCE), a platinum wire counter electrode, and Ag/AgCl (3.0 mol L^{-1} KCl) as a reference electrode. Glassy carbon disk electrode (GCE) with a formal surface area of 0.0314 cm^2 was used as the basal working electrode. A Corning pH–meter, Model 140, with a glass electrode (conjugated with an Ag/AgCl reference electrode, Model 6.0232.100), was used to determine the pH values of the solutions.

The Trypan Blue was purchased from Sigma-Aldrich (Dye content, 60%), and UA, Cys and Tyr were purchased from Sigma-Aldrich. Stock solution of Tyr (1.0×10^{-3} mol L^{-1}) was prepared by dissolving a suitable amount of Tyr in 2 mL of 0.10 mol L^{-1} Hydrochloric acid (HCL) and was diluted to 1 L in a volumetric flask. Stock solution of UA (1.0×10^{-3} mol L^{-1}) was prepared by dissolving a suitable amount of UA in 2 mL of 0.10 mol L^{-1} of NaOH diluted to 1 L in a volumetric flask. Cys (1.0×10^{-3} mol L^{-1}) was prepared by dissolving a suitable amount of the reagent in water. Phosphate buffer solutions (PBS) with different pH levels were prepared by mixing 0.10 mol L^{-1} Na_2HPO_4 and 0.10 mol L^{-1} NaH_2PO_4 solutions at different ratios. The solution pH levels were adjusted by adding 1.0 mol L^{-1} H_3PO_4 and/or NaOH solution.

2.2. Preparation of AuNPs/poly-TrB modified glassy carbon electrode

Prior to electropolymerization, the bare GCE was mechanically polished with alumina powder (Al_2O_3, 0.05 μm). In order to remove any adsorbed substance on the surface of the electrode, it was ultrasonicated in ethanol and doubly distilled water for 5 min. Then, the electrode was dried under nitrogen flow and was ready to use. The electrode was subsequently immersed in a solution containing 0.005 mol L^{-1} TrB and 0.01 mol L^{-1} NaOH, and carried out by cyclic voltammetry (between 0.0 and +1.5 V at 50 mV s^{-1}) for 30. Finally, the resulting electrode, poly-TrB/GCE, was immersed into 0.1 M KNO_3 containing 3.0 mmol L^{-1} $HAuCl_4$ to electro-deposit AuNPs for 120 s, where electrochemical deposition was conducted at −200 mV by a single potential mode. The obtained electrode was donated as AuNPs/poly-TrB/GCE was washed with water, and the scan was activated by several cyclic voltammetry in a potential range between 0 and +0.80 with a scan rate of 100 mV s^{-1} in buffer solution (pH 3.0) until a steady state response appeared to increase its reproducibility. The whole process was conducted at room temperature.

2.3. Preparation of real samples

Serum samples were obtained from the Health Center of Isfahan University of Technology and stored frozen at −20 °C until they were analyzed. Then, an initial centrifugation at 3000 rpm for 10 min was applied to separate the plasma sample. The plasma was deproteinated according to Aly method [27]. 1.0 mL of plasma sample and 2.0 mL acetonitrile as a deproteinated reagent was mixed and centrifuged for 10 min at 2000 rpm. Then, the upper layer of the volume was transferred to a small flask and was evaporated with the stream of nitrogen. After drying, the residue was diluted with phosphate buffer solution of pH 4.0 and was applied for analysis with the proposed method. To remove the matrix effect, standard addition method was utilized to determine Cys, UA and Tyr in these samples.

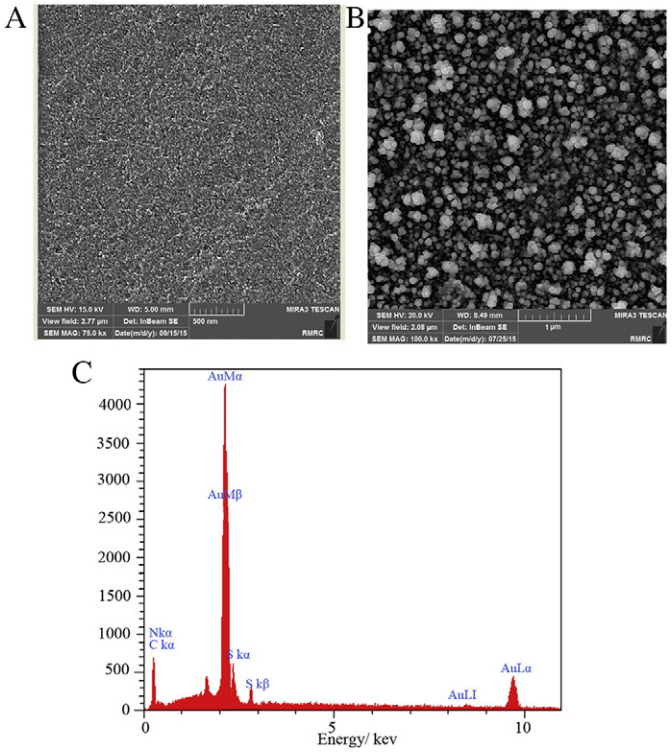

Fig. 1. Scanning electron microscopy image of (A) poly-TrB modified glassy carbon electrode, (B) AuNPs/poly-TrB film modified glassy carbon electrode, and (C) the corresponding EDX spectrum taken from the whole area of (B).