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A high sensitive biosensor based on FePt/CNTs nanocomposite /*N*-(4-hydroxyphenyl)-3,5-dinitrobenzamide modified carbon paste electrode for simultaneous determination of glutathione and piroxicam

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ABSTRACT

This study describes the development, electrochemical characterization and utilization of novel modified *N*-(4-hydroxyphenyl)-3,5-dinitrobenzamide-FePt/CNTs carbon paste electrode for the electrocatalytic determination of glutathione (GSH) in the presence of piroxicam (PXM) for the first time. The synthesized nanocomposite was characterized with different methods such as TEM and XRD. The modified electrode exhibited a potent and persistent electron mediating behavior followed by well-separated oxidation peaks of GSH and PXM. The peak currents were linearly dependent on GSH and PXM concentrations in the range of 0.004–340 and 0.5–550 μmol L^{-1}, with detection limits of 1.0 nmol L^{-1} and 0.1 μmol L^{-1}, respectively. The modified electrode was successfully used for the determination of the analytes in real samples with satisfactory results.

1. Introduction

Glutathione is the most important molecule that need to stay healthy and prevent aging, cancer, heart disease, dementia and more, and necessary to treat everything from autism to Alzheimer's disease. The role of glutathione in the human metabolism includes protection against oxidative stress and detoxification of xenobiotics. In addition, it plays an essential role in many important biological phenomena, including the synthesis of proteins and DNA and protection of cells against free radicals (Ensafi et al., 2008). Studies have shown that the total GSH present in cells may be either free or bound to proteins. The amount of free GSH in blood is indicative of cell protection against oxidative and free radical-mediated cell injury. In addition, GSH levels in blood samples help in diagnosis of c-glutamyl cycle disorders. Its precise determination is, therefore, of utmost importance for diagnostic purposes (Ensafi et al., 2012; Keyvanfard et al., 2013). The determination of GSH has been carried out with various detection techniques such as titrimetry (Nagendra et al., 2002), spectrophotometry (Kamata, et al., 1995; Raggi et al., 1991), spectrofluorimetry (Liang et al., 2002; Zhang et al., 2005; Kandar et al., 2007), high performance liquid chromatography (HPLC) (Katrusiak et al., 2001; Xu et al., 2002), capillary zone electrophoresis (Causse et al., 2000), proton nuclear magnetic resonance (1H NMR) (Rabenstein et al., 1985), enzymatic method (Satoh et al., 1988), flow injection analysis (Ensafi et al., 2008) and electrochemical methods (Raoof et al., 2009a, 2009b; Ensafi et al., 2010).

Piroxicam is a non-steroidal anti inflammatory drug (NSAIDs) with analgesic and anti-pyretic activities (Bavili Tabrizi, 2007). Piroxicam exhibited a rapid and effective response in the treatment of many diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout juvenile rheumatoid arthritis, musculoskeletal disorders, postpartum pain and sport injuries. The most serious reported side effects are gastrointestinal effects, such as ulcer, bleeding ulcers, etc (Damiani et al., 1998).

Also, PXM as NSAIDs can be effective on the biological compounds that presence in the brain especially on concentration of glutathione (Damiani et al., 1998). So, these facts let us assume that piroxicam produce toxic events that are different according to the brain ((El-Sherbiny et al., 2009).

Electrochemical biosensors have been the subject of basic as well as applied research for nearly fifty years. Studies show that

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electrochemical biosensors modified with nanomaterials can be improve electrical signal for important biological compounds with high overpotential and low intensity (Micheli et al., 1993; Ensafi et al., 2011a,2011b,2011c; Sanghavi and Srivastava, 2011, 2010; Sanghavi et al., 2013; Yola et al., 2013). So, much attention has been done for preparation of high selective and sensitive electrochemical biosensors for analysis of biological compounds in biological and pharmaceutical samples such as urine, blood and tablet (Yola et al., 2012; Gupta et al., 2014, 2005, 2013).

According to the above points, it is very important to create suitable conditions for the simultaneous analysis of GSH and PXM in biological samples. However, to best of our knowledge, there is no report on the voltammetric determination of GSH and PXM simultaneously; using modified electrodes. Therefore, in continuation of our studies on the preparation of chemically modified electrodes (Karimi-Maleh et al., 2013; Elyasi et al., 2013; Moradi et al., 2013; Ensafi et al., 2011a,2011b,2011c; Sanati et al., 2014), a novel *N*-(4-hydroxyphenyl)-3,5-dinitrobenzamide-FePt/CNTs carbon paste electrode (NHPDA/FePt/CNTs/CPE) for the voltammetric determination of GSH was investigated using square wave voltammetry. We have also evaluated the analytical performance of the modified electrode for quantification of GSH in the presence of PXM in some real samples.

2. Experimental

2.1. Synthesis of FePt/CNTs nanocomposite

Before introducing the FePt particles onto the surface of the CNTs, the CNTs were treated by boiling the as-received CNTs in mixture of HNO_3:H_2SO_4 for 4 h, rinse with distilled water, dried and ground.

FePt nanoparticles supported on multi-walled carbon nanotubes obtained by mixing stoichiometric of purified MWCNTs, Fe $(acac)_3$ (0.5 mmol), $Pt(acac)_2$ (0.5 mmol) and 1,2 Hexadecanediol (5 mmol) in 40 mL phenylether at room temperature under a flow of N_2 atmosphere, and then the solution was heated at 100 °C for 20 min. Reduction of Fe and Pt atoms in the presence of 1,2 Hexadeconediol have been obtained until starting the reduction of Fe and Pt salts and nucleation of FePt nanoparticles. Afterward the solution was heated to 260 °C for 90 min during the reflux process. After completion of reaction, the black product solution was cooled to room temperature under flow of N_2 atmosphere. Then the product was rinsed with ethanol and hexane solution for 3 times and dried in 200 °C under Ar atmosphere.

2.2. Chemicals

All chemicals used were of analytical reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout.

A 1.0×10^{-2} mol L^{-1} GSH solution was prepared daily by dissolving 0.30 g glutathione in water and dilution the solution to 100 mL with water in a 100-mL volumetric flask. The solution was kept in a refrigerator at 4 °C and in dark. More dilute solutions were prepared by serial dilution with water.

A 1.0×10^{-3} mol L^{-1} PXM solution was prepared daily by dissolving 0.033 g PXM in methanol–water (1:1 v/v) and the solution was diluted to 100-mL with water in a 100-mL volumetric flask. The solution was kept in a refrigerator at 4 °C in dark. More dilute solutions were prepared by serial dilution with water.

Phosphate buffer (sodium dihydrogen phosphate and disodum monohydrogen phophate plus sodium hydroxide, 0.1 mol L^{-1}) solutions (PBS) with different pH values were used.

2.3. Apparatus

Square wave voltammetry (SWV), cyclic voltammetry, electrochemical impedance spectroscopy (EIS) and chronoamperomtry were performed in an electroanalytical system, Autolab PGSTAT 12N, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand linked with a computer (Pentium IV, 1200 MHz) and with Autolab software. The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100 kHz–0.1 Hz was employed. The AC voltage amplitude was 5 mV, and the equilibrium time was 30 min. A conventional three-electrode cell assembly consisting of a platinum wire as an auxiliary electrode and an $Ag/AgCl/KCl_{sat}$ electrode as a reference electrode was used. The working electrode was either a carbon paste electrode (CPE), FePt/CNTs/CPE, NHPDA/FePt/CNTs/CPE or NHPDA/CPE.

2.4. Preparation of the modified electrode

30.0 mg of NHPDA was hand mixed with 870 mg of graphite powder and 100 mg of FePt/CNTs in a mortar and pestle. Using a syringe, 0.55 g of paraffin was added to the mixture and mixed well for 45 min until a uniformly wetted paste was obtained. The paste was then packed into a glass tube. Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper. The unmodified carbon paste electrode was prepared in the same way without adding mediator and NHPDA/FePt/CNTs/CPE to the mixture.

2.5. Preparation of real samples

Human whole blood was obtained from the Kerman Health Center and erythrocytes were separated from whole blood by removing the plasma. Human whole blood (2.0 mL) was first centrifuged for 10 min at 3000 rpm. The supernatant (plasma) was discarded and the rest was mixed with 5 mL of 0.9% NaCl solution. The solution was centrifuged for another 10 min at 4000 rpm and the supernatant (diluted plasma) was again discarded. The washing procedure with NaCl solution was repeated three times in order to remove the plasma almost completely.

The erythrocyte pellets were hemolysed with water (1:1, v/v). For protein precipitation, the hemolysate was mixed with 5-sulfosalysilic acid (10%, m/v) in the ratio of 2:1 (v/v). This mixture was centrifuged in the same condition described above. Then, the supernatant was divided to two parts, one for spectrophotometric determination and another for the proposed electrochemical method. For spectrophotometric measurements of the Ellman, a reference method (Ellman, 1959) was performed which is based on the reaction of glutathione and DTNB (Ellman's reagent), generating 2-nitro-5-mercapto-benzoic acid. The absorbance was monitored spectrophotometrically at 412 nm.

The urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 30 min at 2500 rpm. The supernatant was filtered using a 0.45 μm filter and then diluted 10-times with PBS (pH 6.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. The standard addition method was used for the determination of GSH in real samples.

The tablet solution was prepared by completely grinding and homogenizing seven tablets of GSH, labeled 100 mg per tablet (Chongqing Yaoyou Pharmaceutical Co., Ltd.). Then, 10 mg of each tablet powder was accurately weighed and dissolved in 100 mL water by ultrasonication. After mixing completely, the mixture was filtered on an ordinary filter paper, 10 mL of which was