



Simultaneous electrochemical determination of glutathione and tryptophan on a nano-TiO₂/ferrocene carboxylic acid modified carbon paste electrode

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ABSTRACT

A modified carbon paste electrode was prepared by incorporating TiO₂ nanoparticles and ferrocene carboxylic acid (FCCA) in carbon paste matrix. Electrochemical impedance spectroscopy (EIS) indicates that the TiO₂ nanoparticles efficiently decreased the charge transfer resistance value of electrode and increased the porous microcrystalline structure of it to improve the electron transfer kinetic between FCCa and electrode. FCCa acted as a mediator between the analyte and electrode surface. The electrocatalytic oxidation of glutathione (GSH) and tryptophan (Trp) is individually and simultaneously investigated at the surface of FCCa-TiO₂ modified electrode using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The electrode is able to completely resolve the voltammetric response of GSH and Trp from potentially interfering species, e.g. ascorbic acid (AA) and tyrosine (Tyr), respectively. The linear range of the analytical plot remains constant in binary mixture solutions of GSH and Trp. High sensitivity and selectivity together with very low detection limit of the electrode response make it very suitable for simultaneous and individual determination of trace amounts of GSH and Trp in pharmaceutical and clinical preparations.

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1. Introduction

Glutathione (L-γ-glutamyl-L-cysteinylglycine), GSH is a key physiological antioxidant that not only detoxifies reactive oxygen species directly, but also enhances the functional ability of other crucial antioxidants, such as vitamins E and C [1,2]. Altered levels of GSH in plasma have been implicated in a number of pathological conditions, including Alzheimer's, Parkinson's diseases, diabetes, macular degeneration, and HIV disease [3]. Analytical methods for the determination of GSH frequently rely on liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) coupled with various detectors, such as fluorescence [4], ultra violet (UV)-vis spectroscopy [5], mass spectrometry [6] and electrochemical detector [7]. Fluorescence and UV detections need GSH derivatization. Also, stability for fluorescence method and selectivity for UV detection are not satisfactory; mass spectrometry detection needs special instrument with high cost.

Tryptophan (2-amino-3-(1H-indol-3-yl)-propionic acid), Trp is essential amino acid for humans and a precursor for serotonin (a neurotransmitter), melatonin (a neurohormone), and niacin. It has been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. This compound is sometimes

added to dietary, food products, pharmaceutical formulas due to the scarcely presence in vegetables. Therefore simple, sensitive and less expensive detection of Trp is of great interest. Therefore, various methods have been reported for the determination of Trp [8–10]. Concentration of amino acids in biological samples is low; therefore it is necessary to use a highly sensitive method that provides determination of these analytes at subordinate concentrations. Electrochemical analytic technique is an attractive method due to simplicity, low expense, high sensitivity and possibility of miniaturization. However, there are following problems for the direct electrochemical oxidation of GSH and Trp at common electrodes: (1) the electron transfer reaction of GSH and Trp is slow and high potential is required for determination and (2) strong adsorption of GSH and Trp at the surface of metallic electrode makes GSH and Trp detection unsatisfactory [11,12]. These problems have been solved using modified electrodes [13–16].

On the other hand, ferrocene and its derivatives due to their good stability in solution, rapid responses to many electroactive substances, independent pH, stability in both oxidized and reduced forms, unreactivity with oxygen, regeneration at low potential and having fast electron transfer are the most successful mediators [17,18]. Therefore, we used ferrocene carboxylic acid as a mediator in this work.

However, to the best of our knowledge, electrochemical determination of GSH and Trp using TiO₂ nanoparticles and FCCa modified carbon paste electrode (FCCA-TiO₂MCPE) has not been reported. Recently, there is a considerable interest in using TiO₂

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nanoparticles as a modifier since they have high surface area, optical transparency, good biocompatibility, and relatively good conductivity. In contrast to the broad application in photochemistry [19,20], the studies of nano-TiO₂ in electrochemistry are not energetic. The major barriers involve the low solubility of TiO₂ nanoparticles and the poor stability of the TiO₂ film on the electrode surface. Efforts have been made to obtain modified electrode with nano-TiO₂, e.g. screen printing procedure [21], sol-gel strategy [22], and dispersing nano-TiO₂ with organic solvent [23]. Carbon paste electrodes (CPEs), due to their ease of construction, renewability, and compatibility with various types of modifiers, have been widely used as suitable matrixes for preparation of modified electrodes. Further, they show rather low background current compared to the solid graphite or noble metal electrodes [24]. In recent years, application of the carbon paste electrodes modified with nanostructure showed considerable improvements in electrochemical behavior of biologically important compounds [25,26].

In the present work, we described the preparation of a composite paste by mixing of 20 wt% nano-TiO₂ and fine graphite powder modified with 1 wt% ferrocene carboxylic acid. This modified electrode showed very efficient catalytic activity toward electrochemical oxidation of GSH, decreasing the anodic overpotential, and improving the anodic current of Trp. A complete resolution between differential pulse voltammetry (DPV) peaks of GSH and Trp (with more than 400 mV in the separation of peak potentials) provides a very suitable and effective method for simultaneous determination of these compounds in pharmaceutical and clinical samples.

2. Experimental

2.1. Materials

Ferrocene carboxylic acid (FCCA), GSH and Trp were obtained from Fluka. Solutions of Trp and GSH were prepared in twice distilled water prior to use. Nano-TiO₂ particles were purchased from Sharif University, Tehran, Iran. Phosphate buffered solutions (PBS) 0.1 M for different pH values were prepared by mixing of stock solutions of 0.1 M H₃PO₄, NaH₂PO₄, Na₂HPO₄ and Na₃PO₄. High viscosity paraffin (density = 0.88 g cm⁻³) from Fluka was used as the pasting liquid for the carbon paste electrode. Graphite powder (particle diameter = 0.1 mm) from Merck was used as the working electrode substrate. Potassium chloride from Fluka was used as the supporting electrolyte. The pharmaceutical and biological samples used in this work were obtained from B. Braun Melsungen AG, Germany and Dr. Safiri Medical Diagnostic Laboratory, Iran, Babol-sar, respectively. The solvent used for the electrochemical studies was twice distilled water. All other reagents used were of analytical grade.

2.2. Preparation of working electrode

The modified electrode (FCCA-TiO₂MCPE) was made by dissolving the mixture of 1% (w/w) FCCa, 20% (w/w) nano-TiO₂ and graphite powder in diethyl ether and hand mixing with a mortar and pestle. The solvent was evaporated by stirring. A 1:1 (w/w) resulting mixture and paraffin was blended by hand-mixing until a uniformly wetted paste was obtained. The resulting paste was inserted in the bottom of a glass tube (with internal radius 1.0 mm and 10 cm length). The electrical connection was implemented by a copper wire lead fitted into a glass tube. When necessary, a new surface was obtained by pushing out an excess of paste and polishing it on weighing paper. The nano-TiO₂CPE and FCCa-CPE as unmodified carbon paste electrodes were prepared in the same way but without adding FCCa and nano-TiO₂, respectively.

2.3. Apparatus

Voltammetric measurements (such as cyclic voltammetry and differential pulse voltammetry) were carried out using a computerized potentiostat and galvanostat (μ -Autolab TYPE III, Eco Chemie BV, Netherlands) and electrochemical impedance spectroscopy (EIS) was performed using a potentiostat and galvanostat (Autolab, model PGSTAT30, Eco Chemie, Netherlands) that were connected to a Pentium IV personal computer. The experimental conditions for voltammetric measurements and impedance spectroscopy were controlled with general purpose electrochemical system (GPES) and frequency response analyzer (FRA) softwares, respectively. All electrochemical studies were performed at 25 \pm 2 °C with a three-electrode assembly including a carbon paste (unmodified or modified) as a working electrode, an Ag|AgCl|KCl (3 M) electrode as the reference electrode, and a platinum wire as a counter electrode. Spectrometric measurements were performed with ultraviolet/visible (UV/vis) spectrophotometer JASCO V-570 (PMT) for recording the absorption spectra. A digital pH-meter (Ion Analyzer 250, Corning) with precision of \pm 0.001 was used to read the pH of the buffered solutions.

2.4. Real sample preparation

Blood samples of 5 mL were collected in tubes and their erythrocytes were separated from whole blood by removing the plasma. The blood was centrifuged at 3000 rpm for 15 min at 10 °C and the plasma (above solution) was discarded. The erythrocytes were washed three times with phosphate buffer (pH 7.0) + 0.9% NaCl solution in order to remove the plasma almost completely. The erythrocytes pellets were hemolysed (1:1, v/v) in 1.0 mmol L⁻¹ Na₂H₂EDTA solution. After preparing the hemolysed erythrocytes, 10% (w/v) of 5-sulfosalicylic acid was added to this solution and the resulting solution was centrifuged at 3000 rpm for 10 min. The supernatant was collected for GSH and Trp determination. For the voltammetric measurements, 100 μ L of the hemolysed sample was diluted in 10 mL of phosphate buffer (pH 7.0). For spectrophotometric measurements the method described by Anderson [27] was performed, which is based on the reaction of glutathione and Ellman's reagent (5,5'-dithio-bis-2-nitrobenzoic acid, DTNB), generating 2-nitro-5-mercapto-benzoic acid. This was monitored spectrophotometrically at 412 nm.

3. Results and discussion

3.1. The influence of pH on the electrochemical properties of GSH and Trp

It is well known that the electrochemical behavior of GSH ($pK_{a1} = 2.12$, $pK_{a2} = 3.59$, $pK_{a3} = 8.75$ and $pK_{a4} = 9.65$) [28] and Trp ($pK_{a1} = 2.38$ and $pK_{a2} = 9.39$) [29] are dependent on the pH value of the aqueous solution, whereas the electrochemical properties of ferrocene carboxylic acid/ferrocenium carboxylic redox couple are pH independent. Therefore, we studied the electrochemical behavior of GSH and Trp in 0.1 M phosphate buffered solution with various pHs (2.0 < pH < 12.0) at the surface of FCCa-TiO₂MCPE using cyclic voltammetry for simultaneous determination of GSH and Trp (Fig. 1). This study showed that for both of compounds, the electrocatalytic peak current and peak potential depended on the solution pH. It can be seen that the anodic peak currents of GSH and Trp reach a maximum value at pH 7.0 and then decrease gradually with increasing of pH (Fig. 1A). Therefore, pH 7.0 was taken as the optimum pH for the simultaneous determination of these two compounds.

Also, the anodic peak potential of GSH at the surface of this modified electrode shifts to less positive values with increasing pH of

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