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Article

Multiwall carbon nanotube paste electrode with 3,4-dihydroxy-cinnamic acid as mediator for the determination of glutathione in pharmaceutical and urine samples

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ABSTRACT

A sensitive and selective electrochemical sensor for the determination of glutathione (GSH) was developed using a modified multiwall carbon nanotube paste electrode with 3,4-dihydroxy-cinnamic acid as a mediator. This modified electrode showed very high electrocatalytic activity for the anodic oxidation of GSH. Under the optimized conditions, the electrocatalytic peak current showed a linear relationship with GSH concentration in the range of 0.5–400.0 μmol/L with a detection limit of 0.1 μmol/L GSH. The relative standard deviations for seven successive assays of 5.0 and 25.0 μmol/L GSH were 2.2% and 2.7%, respectively. The modified electrode was used for the determination of GSH compounds in real urine samples.

1. Introduction

Glutathione (GSH) is a biological thiol compound that is the main intracellular tripeptide in mammals. Its role in human metabolism includes protection against oxidative stress and detoxification of xenobiotics [1]. Changes in its concentration in biological fluids or tissues are a useful marker of certain disorders such as leukemia [2], diabetes [3], DNA base damages [4] and in the investigation of some kinds of cancer [5]. A number of methods have been proposed for the determination of GSH including titrimetry [6], spectrophotometry [7,8], spectrofluorimetry [9], high performance liquid chromatography [10–12], capillary zone electrophoresis [13,14], proton nuclear magnetic resonance (^{1}H NMR) [15,16], and enzymatic [17] and electrochemical methods [18–22]. Electrochemical methods have shown significant advantages in the analysis of different compounds in biological and pharmaceutical samples. These advantages are mainly attributable to the simplicity, low cost and relatively short analysis times of these compounds as compared to chromatography and other methods [23]. In recent years, chemically modified electrodes have attracted much notice due to their potential applications in various analyses [24,25].

Carbon nanotubes (CNTs) have a novel type of nanostructure with unique structural electronic and mechanical properties and have been studied extensively since their discovery [26–28]. Research over the past decade has revealed that the CNTs constituted a new form of carbon materials that are finding striking applications in many fields, such as energy conversion and storage [29], chemical actuators [30,31], and chemical sensing [32–36]. In the present work, we described the preparation of a multiwall carbon nanotube paste electrode (MWCNTPE) with 3,4-dihydroxy-cinnamic acid (3,4-DHCA) as a suitable sensor for the electrocatalysis and determination of

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GSH in aqueous buffer solution. The proposed method is selective, sensitive, and fast for the determination of GSH in real samples such as GSH tablets, urine, and hemolyzed erythrocyte.

2. Experimental

2.1. Apparatus and reagents

All the voltammetric measurements were performed using an Autolab PGSTAT 302N, potentiostat/galvanostat (Utrecht, The Netherlands) connected to a three-electrode cell, Metrohm (Herisau, Switzerland) Model 663 VA stand. This was operated by a computer (Pentium IV, 1200 MHz) with the Autolab software. A platinum wire was used as the auxiliary electrode. MWCNTPE and Ag/AgCl/KCl_{sat} were used as the working and reference electrodes, respectively. The electrode prepared with CNTs was characterized by scanning electron microscopy (SEM, Seron Tech. AIS 2100). A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. Spectrally pure graphite powder (particle size < 50 μm) from Merck and multiwall carbon nanotubes (> 90% MWCNTs basis, $d \times l$ = (90–70 nm) × (5–9 μm)) from Fluka were used for the preparation of the carbon paste electrode.

Phosphate buffer (NaH_2PO_4-Na_2HPO_4-NaOH 0.1 mol/L) solutions (PBS) with different pH values were used. All chemicals used were analytic reagent grade purchased from Merck (Darmstadt, Germany) unless otherwise stated. Doubly distilled water was used throughout. GSH was from Fluka.

2.2. Preparation of the electrode

To eliminate any metal oxide catalyst in the nanotubes, the MWCNTs were refluxed in 2.0 mol/L HNO_3 for 12 h, and then washed with twice distilled water and dried at room temperature. Graphite powder (0.900 g) was dissolved in diethyl ether and hand mixed with 0.100 g CNTs in a mortar and pestle. The solvent was evaporated by stirring. A syringe was used to add paraffin to the mixture, which was mixed well for 40 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.

2.3. Preparation of real samples

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

The erythrocyte pellets were hemolyzed 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 under the same conditions described above. Then, the supernatant was divided to two parts: one for spectrophotometric determination and another for the proposed electrochemical method. For spectrophotometric measurements with a reference method [37], the Ellman method was used, which is based on the reaction of GSH and DTNB (Ellman's reagent) to generate 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. The sample (10 mL) was centrifuged for 20 min at 2000 r/min. The supernatant was filtered using a 0.45 μm filter and then diluted 5 fold with PBS (pH 7.0). The solution was transferred into the voltammetric cell for analysis 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 to have 100 mg per tablet (Chongqing Yaoyou Pharmaceutical Co., Ltd., China). 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 subsequently transferred into a 100 mL volumetric flask and diluted to the mark with water. Then, 1.0 mL of the solution plus 4.5 mL of the buffer (pH 7.0) was used for analysis using the standard addition method.

2.4. Optimization of 3,4-DHCA concentration

The influence of 3,4-DHCA concentration on the electrocatalytic oxidation peak current was studied at two different concentrations of GSH at pH 5.0, and in the range of 100.0 to 700 μmol/L 3,4-DHCA. The results showed that by increasing the concentration of 3,4-DHCA up to 500 μmol/L the peak current increased, while higher concentrations of 3,4-DHCA caused a slight decrease in the magnitude of the peak current, which may be due to the formation of 3,4-DHCA aggregates. Therefore, 500 μmol/L 3,4-DHCA concentration was selected for further studies.

3. Results and discussion

3.1. SEM characterization

Figure 1 shows SEM images of the MWCNTPE and carbon paste electrodes (CPE). As can be seen at the surface of CPE, layers of irregular flakes of graphite powder were present that were isolated from each other. After MWCNTs were added to carbon paste, it can be seen that MWCNTs were distributed on the surface of electrode with a special three dimensional structure, indicating that the MWCNTs were successfully assimilated on the MWCNTPE.

3.2. Electrochemistry of 3,4-DHCA