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A high sensitive electrochemical nanosensor for simultaneous determination of glutathione, NADH and folic acid

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ABSTRACT

In the present study, we report electrosynthesis of 4,5-bis(4-chloroanilino)-1,2-benzendiol (BCB) and its application as a selective electrochemical mediator at a surface of carbon paste electrode (CPE) modified ZnO/CNTs nanocomposite as a simple and rapid voltammetric sensor. The sensor showed an efficient catalytic activity for the electro-oxidation of glutathione (GSH), which leads to a lowered overpotential by more than 203 mV compared to unmodified carbon paste electrode. For the mixture containing GSH, nicotinamide adenine dinucleotide (NADH) and folic acid (FA), the electrooxidation signals were well separated. The square wave voltammetry (SWV) currents increased linearly with their concentration at the ranges of 0.006–161, 1.0–650 and 3.0–700 μM, respectively with the detection limits of 0.002, 0.3 and 1.0 μM. Finally, the electrode was successfully applied for the voltammetric determination of analytes in real samples with satisfactory results.

1. Introduction

The fabrication of chemically modified electrode (CME) has been widely reported to improve selectivity and sensitivity for determination of electroactive materials such as amino acids, vitamins, pharmaceuticals, and pollutants. [1–4]. Generally, CME incorporates a thin film, particles of conducting compounds or polymers which are bonded to or coated on the electrode surface to detect the target analytes [5–9].

Nanostructure compounds such as nanosheets, nanoparticles, carbon nanotubes and nanocomposites are being used for numerous bioanalytical applications in CME format [10–14]. Among various nanomaterials, zinc oxide (ZnO) has a great potential due to its numerous exotic properties such as large specific surface area, biocompatibility, high conductivity, ease of fabrication and so on [15–17].

Endogenous thiols such as GSH play important roles in metabolism and cellular homeostasis. These compounds have been found in many living cells, and serve diverse biological functions such as redox regulation and detoxification [18–20]. As an antioxidant, GSH reacts with reactive oxygen species such as H_2O_2 and other active peroxides. Furthermore, some studies have demonstrated that children with autism have decreased glutathione and increased oxidative stress [21–23]. Therefore, GSH measurement is important in many biological matrices such as plasma and hemolyzed erythrocyte. Diverse analytical methods have been reported for the determination of GSH that could be classified into titrimetry [24], spectrophotometry [25], spectrofluorimetry [26–28], high-performance liquid chromatography [29,30], capillary zone electrophoresis [31], flow injection analysis [32] and electrochemical methods [33–36].

NADH, as a cofactor, is involved in numerous enzymatic reactions of NAD^+/NADH dependent dehydrogenases [37]. Some uncontrolled studies have found NADH benefits for patients suffering from Parkinson's disease, Alzheimer's disease, and depression [38–40]. Studies showed that NADH is needed for the regeneration of GSH after its oxidation; also supplementation of NADH may help restoring GSH to its active form. Therefore, the level of GSH is related to NADH concentration in human cells [41]. Due to the abovementioned reasons, simultaneous determination of these compounds in biological samples is very important.

Folic acid (FA, N-[p-{[(2-amino-4-hydroxy-6-pteridinyl)methyl] amino}benzoyl]-L-glutamic acid) is a water-soluble B vitamin, which possesses the considerable biological importance, especially during periods of rapid cell division and growth [42]. FA is not the physiologically active form of the vitamin but it is used as a dietary supplement or for enrichment of food samples [43]. The deficiency of FA will cause serious complications during pregnancy which can result in malformations of the spine, skull and brain [44]. High homocysteine levels are associated with many health problems and may be contributed to the progression of Huntington's disease (HD). FA helps to keep homocysteine levels low by aiding one of the two processing pathways. In the first processing pathway, homocysteine is metabolized to cysteine, which can be converted to glutathione. Since breaking down harmful homocysteine into helpful glutathione is a positive effect of the first pathway, the first pathway is important to HD patients. In the second pathway, homocysteine is converted to methionine, which its biosynthesis depends

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on an enzyme that needs folic acid. By having enough folic acid in the body, we can ensure that homocysteine level will be low enough to the level that does not induce the adverse effect [45].

Considering the abovementioned importance, it is very important to develop a robust analytical method for simultaneous determination of GSH, NADH and FA in biological samples such as urine and blood. The problems encountered using the techniques, such as HPLC and spectroscopy, are either the need for derivatization or the need for time-consuming extraction procedures. Since these techniques have expensive instrumentation and running costs, the use of simpler, faster, and cheaper, yet sensitive, electroanalytical techniques can be interesting alternatives. To best of our knowledge, there has been no report yet for voltammetric determination of GSH, NADH and FA simultaneously in biological samples using modified electrodes. In this work, in continuation of our previous efforts on the preparation of chemically modified electrodes [14,46,47], the application of a new catechol derivative, 4,5-bis(4-chloroanilino)-1,2-benzendiol, as a novel mediator is studied. In this study, we used voltammetry and electrochemical impedance spectroscopy techniques to study the electrochemical behavior of GSH, NADH and FA on a novel ZnO/CNTs nanocomposite paste electrode modified with 4,5-bis(4-chloroanilino)-1,2-benzendiol as a mediator (ZnO/CNTs/BCB/CPE). The results showed that the proposed method is highly selective and sensitive for voltammetric determination of GSH, NADH and FA simultaneously.

2. Experimental

2.1. Chemicals

All chemicals were analytical grade reagents purchased from Merck (Darmstadt, Germany) or Sigma Company unless otherwise stated. The commercial multi-walled CNTs with tube diameters of 20–50 nm obtained from Nanostructured & Amorphous Materials (Houston, TX, USA) were used. Doubly distilled water was used throughout. Phosphate buffer (sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide, 0.1 M) solutions (PBS) with different pH values were used. High viscosity paraffin ($d = 0.88$ kg L^{-1}) from Fluka was used as pasting liquid for the preparation of carbon paste electrodes.

2.2. Apparatus

Cyclic voltammetry, electrochemical impedance spectroscopy, chronoamperometry and square wave voltammetry were performed in an analytical system, μ-Autolab with PGSTAT (Eco Chemie, the Netherlands). The system was operating on a PC using NOVA and FRA 4.9 software. 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 with a ZnO/CNTs/BCB/CPE, ZnO/CNTs/CPE, BCB/CPE and CPE was investigated. X-ray powder diffraction studies were carried out using a STOE diffractometer with Cu-Ka radiation (k = 1.54 Å). A pH-meter (Corning, Model 140) with a double junction glass electrode was used to check the pH of the solutions. Samples for transmission electron microscopy (TEM) analysis were prepared by evaporating a hexane solution of dispersed particles on amorphous carbon coated copper grids. Transmission electron microscopy studies were performed on a model CM10 transmission electron microscope (TEM; Philips).

2.3. Synthesis of ZnO/CNTs

The preparation of ZnO/CNTs catalysts was done in three steps. First, the chemical pretreatment of CNTs is required. A definite amount of CNTs was introduced into 80 cm^3 of nitric acid and sulfuric acid (3:1 in volume) solution, then 20 cm^3 of ethanol was dropped into the solution slowly, and the solution was agitated in a shaker at 70 °C and 200 rpm for 18 h. In the second step, certain amounts of purified CNTs (12 g) were dispersed into distilled water solution of NaOH (0.5 M; 100 mL) by ultrasonication for 20 min. The third step involves the supporting of zinc oxide on CNTs by a direct deposition process. First, 14.8 g $Zn(NO_3)_2 \cdot 2H_2O$ was dissolved in 100 mL distilled water. Under constant magnetic stirring, the solution of $Zn(NO_3)_2 \cdot 2H_2O$ was added drop wise to the solution of CNTs at 50 °C through a dropping funnel. The rate of addition of the salt solution was kept approximately at 15 mL/h. After completion of the precipitation procedure, the mixture was stirred at room temperature for 12 h, washed and filtered continuously in distilled water (pH 7.0), and dried at 130 °C. The solid samples were then calcined at 250 °C for 2 h.

2.4. Electrochemical synthesis of BCB (**4**)

According to a particular procedure, 100 mL of phosphate buffer solution (pH 6.5, 0.20 M) was pre-electrolyzed at the chosen potential (1.0 V vs. $Ag/AgCl/KCl_{3M}$) in an undivided cell; then 0.5 mmol of catechol (**1**) and 0.5 mmol of p-chloroaniline (**2**) were added to the cell. The electrolysis was terminated, when the decay of the current became more than 95%. At the end of electrolysis, the precipitated solid was collected by filtration and washed thoroughly with water. The product was characterized by ^{1}H NMR, ^{13}C NMR spectroscopy.

^{1}H NMR (400 MHz, CDCl3): δ 8.8 (br s, 2H, OH); 7.42 (d, 4H, H-3′ and H-5′); 7.08 (d, 4H, H-2′ and H-6′); 6.08 (s, 2H, H-3 and H-6); 1.51 (br s, 2H, NH) ppm. ^{13}C NMR (100 MHz, CDCl3): δ 129.011; 128.76; 128.52; 123.22; 119.57; 115.03; 96.16 ppm.

2.5. Preparation of the sensor

ZnO/CNTs/BCB/CPE was prepared by hand-mixing of 0.80 g of graphite powder, 0.04 g BCB and 0.16 g ZnO/CNTs plus paraffin at a ratio of 70/30 (w/w) and mixed well for 45 min until a uniformly wetted paste was obtained. The paste was then packed into a glass tube (internal radius 1.8 mm). 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.

2.6. Preparation of real samples

Human whole blood (Healthy man, 29 years old) was obtained from National Health Center (Sari, Iran). The erythrocyte contents were separated from the whole blood by removing the plasma. Human whole blood (2.0 mL) was firstly centrifuged for 20 min at 2500 rpm. The supernatant (plasma) was discarded and the rest was mixed with 5 mL of 0.9% NaCl solution. The solution was centrifuged one more time for 10 min at 2500 rpm and the supernatant (diluted plasma) was discarded. The washing procedure with the NaCl solution was repeated five times, in order 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-sulfosalicylic acid (10%, w/v) in the ratio of 2:1 (v/v). This mixture was centrifuged in the same conditions described above.

Urine samples were stored in a refrigerator immediately after collection from the National Health Center (Sari, Iran). 25 mL of the sample was centrifuged for 30 min at 2500 rpm. The supernatant was filtered using a 0.45 μm filter and then diluted 5-times with PBS (pH 7.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of GSH, NADH and FA.

To prepare tablet solution, seven tablets of GSH, labeled 100 mg per tablet (Chongqing Yaoyou Pharmaceutical Co., Ltd—China) were completely grinded and homogenized. Then, 10 mg of the tablet powder was accurately weighed and dissolved in 100 mL water by ultrasonication. After mixing completely, the mixture was filtered on