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Sensitive and selective determination of uric acid in real samples by modified glassy carbon electrode with holmium fluoride nanoparticles/multi-walled carbon nanotube as a new biosensor

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ABSTRACT

A novel modified glassy carbon electrode with holmium fluoride (HoF_3) nanoparticles (HoFNPs)-multiwalled carbon nanotubes (MWCNTs) is fabricated and then successfully used for the sensitive and selective determination of uric acid (UA) in the presence of ascorbic acid (AA) and dopamine (DA). HoFNPs are successfully attached on the multi-walled carbon nanotubes (MWCNTs) via the intermediate of non-covalent hydrophobic interactions of the MWCNTs surface with sodium dodecyl sulfate (SDS). A detailed investigation by transmission electron microscopy (TEM) and electrochemistry is performed in order to elucidate the preparation process and properties of the nanocomposites. TEM image shows that the particle size of HoFNPs is about 56 nm. Linear calibration plot is obtained over the range 2.0×10^{-7}–5.0×10^{-4} M (0.2–500.0 μM) with a detection limit 1.6×10^{-7} M (0.16 μM) for UA. The modified carbon electrode proves to have good sensitivity, stability and performance for determination UA in the presence of AA, DA in human blood serum and urine samples.

1. Introduction

Uric acid (2,6,8-trihydroxypurine, UA), is main final products of purine metabolism in the human body and it is produced from the cellular breakdown products of the purine nucleosides, adenosine and guanosine [1]. UA in biological fluids plays determining roles not only in human metabolism but also in the central nervous and renal systems. Physiological UA serum levels range from 0.347 to 0.745 mM and urinary excretion is typically 21.0–63.5 mM. It has been shown that abnormal levels of UA in the body are symptom of such diseases like gout, Lesch–Nyan syndrome, kidney damage and cardiovascular [2–4]. Disorders of purine biosynthesis and purine catabolism are generally considered due to the abnormal concentrations of UA dissolved in human urine and/or blood [5,6]. Many epidemiological studies have suggested that serum UA is also a risk factor for cardiovascular disease [7]. There are two existing methods for determination of UA in biological samples. The first method, reductive method is non-specific and involves the oxidation of uric acid with phosphotungstate reagent to allantoin with resultant blue coloring of tungstate solution. The second method, enzymatic method is a specific method. It involves the catalytic oxidation of uric acid with the enzyme uricase to allantoin with the formation of hydrogen peroxide [8]. The peroxide, the concentration of which is directly proportional to the concentration of uric acid, could then be determined by a number of methods. But these methods have several disadvantages such as long reaction times, high detection limit and operational cost. Therefore, it is of clinical significance to develop simple and effective methods for the determination of UA. Among the various analytical methods, electrochemical analysis has been proved to be a very promising approach for UA detection. The major problem for the electrochemical detection of UA is that, at conventional solid electrodes, UA and the coexisting interferent ascorbic acid (AA) and dopamine (DA) are oxidized at the close potentials, resulting in overlapped voltammetric responses. Moreover, electrochemical oxidation of UA at bare electrodes usually requires very high overpotential and the electrode surface suffers fouling effect due to the accumulation of oxidation products. In order to overcome these difficulties, many efforts have been carried out for developing novel materials for modifying the electrode and various modified electrodes with polymer film [9], nanoparticles [10,11] metal oxide nanoparticles [12], carbon nanomaterials [13] and ionic liquid [14] have been constructed.

In this work, we present for the first time the application of a modified GCE using immobilizing of HoFNPs on the MWCNTs for determination of UA in the presence of AA, DA. The analytical performance of this sensor for determination of uric acid in the presence of ascorbic acid and dopamine in urine and human serum samples is evaluated by cyclic voltammetry.

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2. Experimental

2.1. Reagents and materials

Multiwall carbon nanotubes (MWCNT), with nanotube diameters, OD = 20–30 nm, wall thickness = 1–2 nm, length = 0.5–2 μm and purity of >95% was purchased from Aldrich. Holmium oxide (Ho_2O_3) was purchased from Prolabo. Uric acid (UA) and Dopamine (DA) were obtained from Merck and used as received. Ascorbic acid (AA) was purchased from Sigma–Aldrich and used as received. Aqueous solutions were prepared with doubly distilled water (DDW). The stock solutions of AA (0.01 M) and DA (0.01 M) were freshly prepared by dissolving ascorbic acid and dopamine hydrochloride in doubly distilled water (DDW), purged with pure nitrogen gas (99.999%) before investigations. The stock solution of UA solution (0.01 M) was prepared by dissolving the solid in a small volume of 0.1 mol L^{-1} NaOH solution and diluted to reach desired concentration. Chitosan (2-amino-2-deoxy-(1–4)-β-D-glucopyranose), a natural polymer, with medium molecular weight, 400,000 Da, was purchased from Fluka and used without further purification. Acetic acid was diluted to a 1% aqueous solution before use. A solution of chitosan (2 mg ml^{-1}) in 1% acetic acid solution was prepared, due to the poor solubility of chitosan, the mixture was stirred to dissolve completely and kept overnight, and the solution was then filtrated through 0.22 μm Millipore syringe filters to remove any impurity before use. Dichloroacetic acid (pK_a = 1.26) was used for the preparation of buffer solutions between pH 1.0 and 1.8. This buffer was denoted DCAABS. The desired pH was achieved with 0.1 M NaOH solution. Similarly, chloroacetic acid (pK_a = 2.87) for pH between 1.8 and 3.8 and acetic acid (pK_a = 4.76) for pH between 3.8 and 5.6 were used. These buffers were denoted CAABS and AABS. The electrolyte solutions were deoxygenated with nitrogen bubbling before each voltammetric experiment. All experiments were performed under nitrogen atmosphere at room temperature. Fresh urine and serum samples were obtained from the Omid Clinical Laboratory (Zahedan, Iran) without any pretreatments.

2.2. Instrumentation

Electrochemical measurements were carried out with an SAMA500 Electroanalyser (SAMA Research Center, Iran) controlled by a personal computer. All electrochemical experiments were carried out in a conventional three-electrode cell at room temperature. A platinum electrode and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. TEM images were taken using a Philips CM120 transmission electron microscopy with 2.5 Å resolution.

2.3. Preparation of working electrodes

In a typical synthesis, the holmium chloride stock solutions of 0.2 M were prepared by dissolving the corresponding Ho_2O_3 in hydrochloric acid at elevated temperatures [15]. The MWCNTs were dispersed by ultrasonication for 3 h in a 1 wt.% sodium dodecyl sulfate (SDS) aqueous solution to modify the MWCNTs surface. After further washing and drying, 100 mg MWCNTs was sonicated in 10 mL of $HoCl_3$ (0.2 M) and added to 20 mL of aqueous solution containing 2 mmol of trisodium citrate (Cit^{3-}) to form the Ho–Cit^{3-} complex. After vigorous stirring for 30 min, 30 mL of aqueous solution containing 25 mmol of $NaBF_4$ was introduced into the solution. The *p*H of the mixture was adjusted to 1 with diluted HCl (1 M). After additional agitation for 15 min, the as-obtained mixing solution was transferred into a Teflon bottle held in a stainless steel autoclave, sealed, and maintained at 180 °C for 24 h. As the autoclave cooled to room temperature naturally, the precipitates were separated by centrifugation, washed with ethanol and deionized water in sequence, and then dried in air at 80 °C for 12 h. This mixture was denoted MWCNT–HoFNPs. The 20 mg of MWCNT–HoFNPs was added to 3 mL of chitosan 1% solution (CH) and the resulting mixture was dispersed with the help of ultrasonic agitation for 2 h to form a MWCNT–HoFNPs–CH suspension. Next, the GC electrode was transferred to the electrochemical cell for further cleaning and activation by using cyclic voltammetry between −1.5 and +1.5 V at a scan rate of 100 mV s^{-1} in 1.0 mol L^{-1} of freshly prepared deoxygenated H_2SO_4 until a stable cyclic voltammetric profile (≈15 times) was obtained and was then used. The clean GC electrode was coated by casting 5.0 μL of the MWCNT–HoFNPs–CH suspension and dried under infrared radiation. This modified GC electrode was denoted as GC/MWCNT–HoFNPs–CH. When not in use, the modified electrode was stored in DDW. The GC/MWCNT–CH was also prepared with the same method.

3. Results and discussion

3.1. TEM characterization of HoFNPs and MWNT–HoFNPs

TEM images of the HoFNPs–CH and MWNT–HoFNPs–CH samples are shown in Fig. 1. Fig. 1a shows TEM image of the prepared HoFNPs, with an average diameter of around 56 nm based on the TEM measurement out of 30 nanoparticles. Also, it can be seen from Fig. 1b, that the HoFNPs are attached to the most of MWCNTs.

3.2. Electrocatalytic investigation of UA in presence of AA and DA

Fig. 2 displays the CVs of a mixture of UA in presence of AA and DA in 0.10 M DCAABS with pH 1.0 at bare GC (BGC), GC/MWCNT–CH and GC/MWCNT–HoFNPs modified electrodes. BGC electrode showed an oxidation peak for DA and a weak and broad oxidation peak for AA–UA at 0.575 and 0.681 V, respectively in the mixture. It is difficult to locate the oxidation potential of AA and UA from Fig. 2a (CV 1) by using the BGC electrode. Furthermore, in the subsequent cycles, the oxidation peak of DA was shifted to positive and it was almost merged with the oxidation potential of AA–UA. This revealed that the bare GC electrode is not suitable for the stable determination of UA in presence of AA and DA.

As it can be seen from Fig. 2a (CV 2 and 3), when GC/MWCNT or GC/MWCNT–HoFNPs were used as the working electrodes, the detection sensitivity was improved significantly and effective separation of the anodic peaks of UA in presence of AA and DA was obtained. Based on Fig. 2a (CV 2), the GC/MWCNT showed an enhancement of the oxidation peak currents of AA, DA and UA in three well-defined at potentials 0.265, 0.455 and 0.616 V in CV, respectively. In addition, the oxidation peak of AA, DA and UA shifts to negative potentials and leads to more peak separation between AA–DA and DA–UA were 0.161 and 0.190 V, respectively. Fig. 2a (CV 3) exhibited the voltammograms of UA in presence of AA and DA at GC/MWCNT–HoFNPs electrode. It could be seen from Fig. 2a (CV 3) that at GC/MWCNT–HoFNPs electrode in pH 1.0 DCAABS, the oxidation peak potential of AA, DA and UA appeared at 0.241, 0.422 and 0.605 V and the peak separation between AA–DA and DA–UA were 0.181 and 0.183 V, respectively. The observed potential difference is more than enough to determine the concentration of UA in the presence of AA and DA and also for simultaneous determination of AA, DA and UA. The oxidation potentials of AA, DA and UA at GC/MWCNT–HoFNPs electrode shifted by about 24, 33 and 11 mV toward the negative values compared with that at a GC/MWCNT electrode. Also, the GC/MWCNT–HoFNPs showed an enhancement in the oxidation peak currents of UA in presence of AA and DA. This suggested that HoFNPs was a dominating participator in the electrocatalytic activity of UA in the presence of AA and DA. These