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Simultaneous determination of ascorbic acid, adrenaline and uric acid at a hematoxylin multi-wall carbon nanotube modified glassy carbon electrode

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ABSTRACT

In this study, multi-wall carbon nanotubes (MWCNT) are evaluated as an immobilization matrix for the construction of a modified electrode based on hematoxylin electrodeposited on MWCNT immobilized on the surface of a glassy carbon electrode, GCE. The results show that the reversibility of hematoxylin is significantly improved at a MWCNT modified GCE in comparison with GCE alone. Hematoxylin MWCNT modified GCE (HMWCNT-GCE) shows an excellent electrocatalytic activity for adrenaline (AD) oxidation, with a diminution of the electrode overpotential of about 395 mV. The detection limit of 0.024 μM and two linear calibration ranges of 0.2–78.3 μM and 78.3–319.7 μM are obtained for AD determination at HMWCNT-GCE surface using a differential pulse voltammetric method. This modified electrode is found quite effective not only in detection of ascorbic acid (AA), AD, and uric acid (UA), but also in simultaneous determination of each component in a mixture. HMWCNT-GCE has been applied to determination of AD in an adrenaline injection and of UA in a human urine sample with satisfactory results.

1. Introduction

Ascorbic acid (AA), adrenaline (AD) and uric acid (UA) are biochemical compounds which play important roles in various biological processes. Ascorbic acid (AA), known as vitamin C, has various physiological and pharmacological functions, as in collagen synthesis [1], intestinal absorption of iron [2], and drug metabolism [3]. It also acts as an important biological antioxidant [4]. Owing to the wide uses of AA in canned fruit, vegetables, animal foods and drugs, many analytical techniques are available for its determination in different matrices and at different levels [5]. These techniques include HPLC [6], spectrophotometry [7], liquid chromatography [8], capillary electrophoresis [9], chemiluminescence [10] and electrochemical methods [11–16].

Adrenaline (AD), [1-(3,4-dihydroxyphenyl)-2-methyloaminoethanol], plays important roles as a neurotransmitter and a hormone. It exists as an organic cation in the nervous tissue and biological body fluid. Many diseases are related to the changes of its concentration in the body fluid [17]. Also, many phenomena are related to the concentration of AD in blood as well as in urine. Therefore, it is very important to develop sensitive and selective analytical methods for the detection of AD in biological fluids [18]. For this purpose, a number of methods have been applied to determine AD, such as spectrophotometry [19], fluorimetry [20], liquid chromatography [21], capillary electrophoresis [22], chemiluminescence [23], electrochemiluminescence [24], and electrochemical detection with various modified electrodes [25–27]. The main problem of AD determination by an electrochemical method in vivo is the interference of AA, which is usually present in high concentrations and can be oxidized at a potential close to that of AD. In addition, the electrooxidation of AD at bare electrodes requires high over-potentials.

Uric acid (UA), (2,6,8-trihydroxypurine), is the final product of purine metabolism in the human body [28]. It is one of the major parameters monitored in urine and in blood. Uric acid concentration changes are associated with the altered metabolism of purines that are related to numerous illnesses and physiological disorders [29]. Therefore, its determination in physiological fluids is necessary in the diagnosis and treatment of diseases such as gout, hyperuricemia, heavy hepatitis, and Lesch–Nyhan syndrome [30]. Uric acid is also a marker for renal failure as well as toxicity. Most analytical methods applied in routine clinical analysis, including uric acid assays, use an optical detection [31]. Therefore, the determination of UA with a simple method is essential because it serves as a marker for the detection of the above diseases. In voltammetric methods, the oxidation potentials of UA and AA, at the most usual electrode surface, are too close to be simultaneously determined. Therefore, it is important to develop simple, rapid and sensitive methods for their simultaneous determination in routine analysis. To achieve this purpose, various modified electrodes have been used for UA determination in the presence of AA [13,32–35].

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In all cases, the chemical modifications of bare electrodes with suitable redox active thin films have been made for the design and development of electrochemical sensors. In practice, electrode surface modification has been tried as a means to reduce the overvoltage and overcome the slow kinetics of many electrode processes [12–16,25–27,32,33–44]. Also, sometimes modified electrodes have been used to separate the electroanalytical signals of the solution components which, at bare electrode surfaces, appear at a potential close to each other [13–15,32,34,35,37–40,42,43,45].

AA, AD, and UA are compounds of great biomedical interest, playing a crucial role in human metabolism. They usually coexist in the extra cellular fluid of the central nervous system and serum. Thus, the individual or simultaneous determination of neurotransmitter of AD and coexisting species, especially AA and UA, is an important issue not only in the field of biomedical chemistry but also in diagnostic and pathological research. We have previously reported that a coumestan derivative [42], norepinephrine [15], tetrabromo-p-benzoquinone [14], and oracet blue [43] modified electrodes could be successfully used to simultaneously determine NADH + UA, AA + UA and AA + DA + UA mixtures. All these reports have their advantages and limitations. Thus, it is necessary to have further efforts for the development of simple, rapid, selective, and sensitive electrochemical modified electrode that can improve the simultaneous detection of different species in the presence of each other.

Also, there are a few papers regarding the electrocatalytic and electrochemical activity of hematoxylin [46–48]. Hematoxylin, as an important biological molecule, is one of the most bioactive flavonoids. Flavonoids are a large family of naturally compounds which widely distributed in various plants [49]. In this study, we report for the first time that hematoxylin multi-wall carbon nanotube modified glassy carbon electrode (HMWCNT-GCE) not only exhibited a strongly catalytic activity for the oxidation of AD, but also resolved the voltammetric responses of AA, AD, and UA compounds into individual signals. Our findings indicate that HMWCNT-GCE has several distinct advantages including extraordinary stability, good reproducibility, wide linear concentration ranges, technical simplicity, high surface charge transfer rate constant, and good detection limit for AD. Also, the modified electrode exhibits a noticeable ability for simultaneous determination of AA, AD, and UA in real samples. Thus, the modified electrode can be of a significant attraction in biological research.

2. Experimental

Ascorbic acid (AA), uric acid (UA), adrenaline (AD), hematoxylin, and the other reagents were obtained from Merck Company and used as received. Multi-wall carbon nanotubes (10–20 nm in diameter, length of 5–20 μm, purity of 95%) were purchased from NanoLab Inc. (Brighton, MA). All the chemical reagents used were of analytical grades. An injection solution of AD (from Darou Pakhsh, Iran) was purchased in a local drugstore. All the solutions were prepared with doubly distilled water. The buffer solutions (0.1 M) were made up from $H_3PO_4 + NaH_2PO_4$, and the pH was adjusted with 0.1 M H_3PO_4 and 2.0 M NaOH. AA, AD, and UA solutions were prepared just prior to use.

All the electrochemical experiments were carried out using an Autolab potentiostat PGSTAT 30 (Eco Chemie Utrecht, Netherlands) equipped with GPES 4.9 software. The cell used was equipped with a hematoxylin multi-wall carbon nanotube modified glassy carbon electrode (HMWCNT-GCE) as a working electrode, a platinum electrode as an auxiliary electrode, and a saturated calomel electrode (SCE) as a reference electrode. All the potentials in the text are quoted versus this reference electrode. A personal computer was used for data storage and processing. The pH was measured with a Metrohm model 691 pH/mV meters.

The preparation of MWCNT modified GCE (MWCNT-GCE) was performed by mechanically polishing a glassy carbon electrode with 0.05 μm Al_2O_3 in water slurry, electrochemically activating it in a 0.1 M sodium bicarbonate solution, and placing 5 μL of DMF-MWCNT suspension (1 mg/1 mL) onto the activated GCE surface. Finally to prepare HMWCNT-GCE, MWCNT-GCE was placed in a 0.1 M phosphate buffer solution (pH 7.0) containing 0.10 mM hematoxylin. It was modified by eight cycles of potential scan rate between −0.4 V and 0.5 V at 50 mV s^{-1}. To fabricate hematoxylin modified GCE (HMGCE), the activated GCE was placed in a 0.1 M phosphate buffer (pH 7.0) containing 0.10 mM hematoxylin. It was modified with the same procedure that was described for HMWCNT-GCE.

3. Results and discussion

3.1. Characterization of HMWCNT-GCE

Fig. 1 shows the cyclic voltammograms of HMWCNT-GCE (Fig. 1A) and HMGCE (Fig. 1B) in a 0.1 M phosphate buffer solution (pH 7.0) as the supporting electrolyte at potential scan rates ranging from 25 mV s^{-1} to 100 mV s^{-1}. When the potential was scanned between 50 mV and 220 mV, a surface immobilized redox couple with a formal potential ($E^{0\prime}$) value of 125 mV was observed for HMWCNT-GCE. In addition, the formal potential, $E^{0\prime}$, is almost independent of the potential scan rate for sweep rates ranging from 15 mV s^{-1} to 6000 mV s^{-1}, suggesting facile charge transfer kinetics over this range of potential sweep rate. The formal potential is obtained from equation $E^{0\prime} = E_{pa} - \alpha\ (E_{pa} - E_{pc})$ [50], considering $\alpha = 0.57$ (see Supporting information). Also the peak-to-peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) is small, about 25 mV for sweep rates below 100 mV s^{-1}. However, for sweep rates above 100 mV s^{-1}, the peak separation begins to increase, indicating the limitation arising from the charge transfer kinetics. But, as it can be seen in Fig. 1B about HMGCE, there exist a surface-confined redox couple with lower peak currents, higher peak separation (70 mV), and low background currents. Comparing the obtained results in Fig. 1A and B, one can see that the reversibility of hematoxylin at HMWCNT-GCE surface is significantly improved. Also, we concluded that MWCNT increases the surface area of the modified electrode. So the background voltammetric response and capacitances of the MWCNT-coated surface are higher than those of the bare surface.

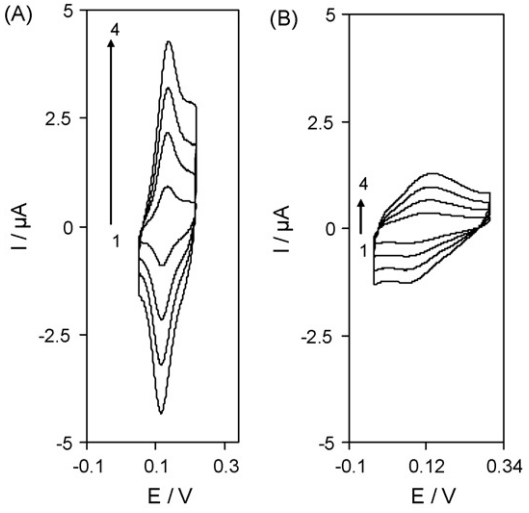

Fig. 1. Cyclic voltammetric responses of (A) HMWCNT-GCE and (B) HMGCE in a 0.1 M phosphate buffer solution (pH 7.0) at different scan rates. Numbers 1–4 correspond to 25 mV s^{-1}, 50 mV s^{-1}, 75 mV s^{-1} and 100 mV s^{-1} respectively.