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Poly-xanthurenic acid modified electrodes: An amperometric sensor for the simultaneous determination of ascorbic and uric acids

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ABSTRACT

The poly-xanthurenic acid (poly-Xa)/multi-walled carbon nanotube (MWCNT)/glassy carbon modified electrode (poly-Xa/MWCNT/GCE) was successfully applied for the simultaneous determination of ascorbic (AA) and uric (UA) acids. Cyclic voltammetric results show that the modified electrode displays an excellent eletrocatalytic activity toward the oxidation of AA at a low potential (0.05 V *versus* Ag/AgCl) and anodic peak resolution (ΔE_{pa}), close to 300 mV *versus* Ag/AgCl, in solutions containing AA and UA. Values in the order of 10^6 L mol^{-1} s^{-1} were obtained for the kinetic constant k_{cat} for the catalytic oxidation of AA, upon evaluation by cyclic voltammetry, chronoamperometry and RDE. Furthermore, the sensor provided limits of detection of 0.19 μmol L^{-1} and 0.31 μmol L^{-1} for AA and UA, respectively. The sensor was applied for the simultaneous determination of AA and UA in urine samples and the average recoveries for these samples were 99.9 (±2.5)% and 99.9 (±2.6)%, respectively.

1. Introduction

Ascorbic (AA) and uric (UA) acids usually coexist in real biological matrices, thus, the development of a selective and sensitive method for their simultaneous determination is highly desirable for analytical and diagnostic applications. AA is a soluble vitamin present in many biological systems and in multivitamin preparations, commonly used as antioxidants [1]. AA has been used for the prevention and treatment of common cold, mental illness, infertility, cancer, AIDS and others [2,3]. Furthermore, often coexisting with AA in biological fluids, such as blood and urine, UA is a primary end product of the purine metabolism and its abnormal level is, normally, indicative of some diseases like gout, hyperpiesia, and Lesch–Nyhan disease [4–7].

Electrochemical determination methods of AA and UA have received much attention because they can be more selective, less expensive and faster. Simultaneous detection of AA and UA is necessary not only in the field of biomedical chemistry but also for diagnostic and pathological research. However, the direct determination on ordinary (for instance, carbon and metal) electrodes is still difficult, because AA and UA undergo oxidation at an overlapping potential and electrode fouling takes place due to the adsorption of oxidation products [8–10]. To overcome these problems, various modified electrodes have been built and used. Ruthenium oxide-modified electrode could attain the capability for simultaneous determination of AA in the presence of UA [11]. More recently, poly(4-amino-1,1′-azobenzene-3,4′-disulfonic acid)-coated electrode has been described for the selective detection of UA in the presence of AA [12]. Pt–Au hybrid film-modified electrode [13], pyrolitic graphite modified electrode [14], zinc oxide composite film [15], palladium nanoparticle-loaded carbon nanofibers [16], surfactant-induced iron(II) phthalocyanine [17] and 4-nitrophthalonitrile modified carbon paste electrode [18] have been reported for the simultaneous determination of AA and UA. Various voltammetric techniques using electrodes modified by materials such as metal NPs [19,20], carbon nanostructures [21,22] and polymers [23] have been proposed to improve detection of AA in the presence of UA. However, all of the reported modified electrodes have their advantages and limitations, such as, low sensitivity, high detection and quantification limits and poor stability. Thus, it is necessary to have further efforts for the development of

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simple, rapid, selective, and sensitive electrochemically modified electrodes that can improve the simultaneous detection of different species in the presence of each other and other interferents.

Recently, we reported the preparation, characterization and application of a simple and efficient poly-xanthurenic acid (Xa)/multiwalled carbon nanotubes (MWCNT)/modified glassy carbon electrode (GCE) based on the electropolymerization of Xa (poly-Xa/MWCNT/GCE) for the electrocatalytic oxidation of NADH [24]. Based on our work, Zhang et al. prepared poly-xanthurenic acid/Fe_2O_3 membranes and applied to DNA sensing [25]. Furthermore, one of the reasons for choosing the MWCNT refers to the versatility of the nanostructured platform, like (i) high electroactive surface area [26], (ii) low charge transfer resistance [27–29], (iii) possibility of functionalization [30–35] and (iv) particularly, in our work, it hampers the blocking effect of the polymer that makes the charge transfer to the analyte, more difficult, as recently reported [24,30].

In the present case, poly-Xa/MWCNT/GCE was investigated toward oxidation for the simultaneous determination of AA and UA.

2. Experimental

2.1. Chemicals and solutions

All the chemicals were of analytical grade and used as received, with no further purification. Multi-walled carbon nanotubes (MWCNT, >95%, 7–15 nm × 0.5–10 μm), AA, UA and xanthurenic acid (Xa) (~96%, Xa) were purchased from Sigma–Aldrich, St. Louis, USA. All the solutions were freshly prepared, prior to each experiment.

2.2. Construction of the sensors and electrochemical measurements

GCE (3 mm in diameter) was manually polished with alumina powder (Al_2O_3, 1 μm) and rinsed with Milli-Q water. After cleaning the electrode, a suspension was prepared by sonication, mixing 2.0 mg of MWCNT and 1 mL of DMF. Two μL of this suspension were then deposited directly upon the GCE surface and dried at 80 °C to form a MWCNT on the GCE surface (MWCNT/GCE) [24].

The electroactive system was electrogenerated *in situ* from Xa oxidation, after cycling (10 scans) in the potential range of −0.5 to +1.0 V *versus* Ag/AgCl, at 50 mV s^{-1}, in a solution of Xa (120 μmol L^{-1}) in 0.1 mol L^{-1} PBS (pH 5.5). Prior to the electropolymerization experiments, PBS was bubbled with nitrogen for 10 min [24].

The voltammetric measurements were performed in an Echo Chemie Autolab PGSTAT-30 potentiostat (Utrecht, The Netherlands). All the measurements were run using an electrochemical cell with three electrodes, with an Ag/AgCl electrode as reference, a Pt wire as auxiliary and an unmodified or modified glassy carbon, in the latter case, with MWCNT/GCE and poly-Xa/MWCNT/GCE as working electrodes. The actual surface area (0.10 cm^2) of the poly-Xa/MWCNT/GCE electrode was estimated using 1 mmol L^{-1} $Fe[(CN)_6]^{3-/4-}$ in 0.1 mol L^{-1} KCl, employing the Randles Sevcik theory as earlier reported [25].

2.3. Real samples analyses

The utilization of the proposed modified electrode in the analysis of real samples was also investigated by a direct analysis of AA and UA in human urine obtained from volunteers. At first, a human urine sample was diluted 50 times with a 0.1 mol L^{-1} phosphate buffer (pH 7.0). Then the urine samples were spiked with 50 μmol L^{-1} of AA and UA.

3. Results and discussion

3.1. Electrocatalytic oxidation of AA and UA

The eletrocatalytic activity of the poly-Xa/MWCNT/GCE was evaluated toward the oxidation of AA and UA. In Fig. 1A, the cyclic voltammogram (a) represents poly-Xa/MWCNT/GCE which has as a mediator, the already reported polymer-based quinone/hydroquinone system [24]. When a solution of AA is added, the oxidation peak, E_{paAA} = 0.05 V *versus* Ag/AgCl significantly increased with the concomitant disappearance of the reduction peak (Fig. 1A (a and c)). However, with the addition of the UA solution, a catalytic effect is not observed, and the UA oxidation occurs at E_{paUA} = 0.35 V *versus* Ag/AgCl (Fig. 1A (b)). To confirm the catalytic activity of the modified electrode toward AA oxidation, AA and UA solutions were added simultaneously (Fig. 1A (d)). The analysis of Fig. 1A (d) allows us to suggest the catalytic nature of AA oxidation with no effect on the UA oxidation. Furthermore, the difference between the two peak potentials is close to 300 mV *versus* Ag/AgCl, large enough to allow the selective and simultaneous determination of AA and UA, in their binary mixture.

Fig. 1B and C display control experiments: firstly, unmodified GCE where the oxidation potential peaks of AA and UA are very closed (Fig. 1B (b and c)). It is practically impossible to analyze the contribution current of each compound in mixture (Fig. 1B (d)). However in Fig. 1C, with GCE modified with only carbon nanotubes (MWCNT/GCE), E_{paAA} and E_{paUA} are now slightly separated (Fig. 1C (b) and (c)), more evident in Fig. 1C (d) with AA and UA together. The electropolymerization of Xa on the GCE forms a non electroactive film which blocks the electrode surface and does not allow AA and UA oxidations (figure not shown). This result shows a synergistic effect between the carbon nanotubes and the surface modified Xa polymer film toward AA catalytic oxidation and simultaneous determination of AA and UA. This behavior may be explained by a faster electron-transfer rate in the chemical modified surface.

To get mechanistic details of the catalytic reaction with AA, cyclic voltammetry at various scan rates was performed (Fig. 2A). The oxidation peak potential had moved toward more positive values and a reduction peak appeared. The peak current was shown to be proportional to $\nu^{1/2}$, as expected for a diffusion-controlled redox process (Fig. 2B). Furthermore, the normalized peak current by the square root of the scan rate ($I_p/\nu^{1/2}$) plotted *versus* the scan rate shows characteristic of an electrochemical–chemical catalytic process (EC′) (Fig. 2C) [27]. A mechanistic proposal for AA oxidation depends on the reduction of the quinonoid group present in the polymeric structure of poly-Xa (Fig. 3). Considering the behavior of the peak potential with the pH solution, between 5.0 and 8.0, the relationship is close to 0.059 (n_p/n_{e-}) V/pH where $n_p = n_{e-}$ was obtained, so, the number of protons involved in this process should be equal to the number of electrons, as showed by Kubota et al. and expected to a quinone/hydroquinone system [18].

3.2. Chronoamperometric studies

The double step chronoamperometry was applied for the investigation of the electrocatalytic process on poly-Xa/MWCNT/GCE. Fig. 4A shows a series of well-defined chronoamperograms of poly-Xa/MWCNT/GCE, in the presence of various AA concentrations. As can be observed in Fig. 4A, there is no net cathodic current corresponding to the reduction of the mediator in the presence of AA, while the forward and backward potential step chronoamperograms for mediator in the absence of AA show very symmetrical