Evidence of an EC' mechanism occurring during the simultaneous assay of ascorbic and uric acids on poly(3,4-ethylenedioxythiophene) modified gold microsensor

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A B S T R A C T

A voltammetric microsensor has been developed for the simultaneous assay of ascorbic (AA) and uric (UA) acids in aqueous solution. The electrode surface has been modified by means of electropolymerized conductive poly(3,4-ethylenedioxythiophene) (PEDOT) organic films. The anodic peak potential separation between both acids was more than 300 mV. The sensitivity of the microsensor for UA was found to be dependent on the presence of AA in the mixture. By using square wave voltammetry (SWV), it increased from 77.5 mA mM^{-1} cm^{-2} without AA to 86.2 mA mM^{-1} cm^{-2} with AA 1 mM. An EC' catalytic mechanism was highlighted, inducing the regeneration of reduced UA by AA at the vicinity of the electrode surface.

Keywords:
Electrochemical microsensor
PEDOT film
Ascorbic and uric acids assay
EC' mechanism

1. Introduction

The simultaneous detection and assay of ascorbic (AA) and uric (UA) acids remain of critical interest, not only for biological researches but also for routine analysis, as they coexist in several physiological liquids (serum, urine, tears, and cerebrospinal fluids). At physiological levels, AA is a powerful water soluble antioxidant and is vital to immune response [1] while extreme AA levels can cause gastric irritation, diarrhea and renal problems [2]. UA is the major final product of purine metabolism in human body. High levels of UA are indicative of gout [3] and represent a risk factor for cardiovascular diseases [4]. Both ascorbic AA and UA are therefore considered as biochemical markers in a lot of pathologies (neonatal hypoxia, coronary heart diseases…).

Among the numerous methods dedicated to their qualitative and quantitative determination, electrochemical techniques present several advantages, such as low cost materials, simple experimental protocols, short time analysis and good accuracy [5]. However, the electrochemical detection of AA and UA on unmodified electrodes results in poor selectivity since their oxidation takes place at very close high overpotentials [6].

To overcome this problem, numerous works have been devoted to the design of chemically modified electrodes. These interfaces introduce electrocatalytic properties or specific molecular interactions which proved to be efficient to reduce overpotentials and to separate AA and UA anodic signals. Several electrode modification processes have been successfully tested [7–12]. However a very few number of these studies focused on the simultaneous detection and determination of AA and UA under similar concentrations [13–15]. For the greatest part of these papers, two situations are generally studied: the first considers AA and/or UA as interfering species, particularly for the assay of dopamine [16–19]; in the second condition, the assay of UA is performed in samples containing AA in large excess [20–23]. However these conditions do not reflect the healthy human situation: the concentration of AA in blood serum is one order of magnitude lower than that of UA (34–79 μM and 180–420 μM respectively [24,25]). Consequently AA was just detected but not assayed in most of these studies while its quantification represents a task of interest as well important as UA in clinical analysis.

We recently developed a voltammetric microsensor that simultaneously determined both AA and UA concentrations in aqueous standard solution and in healthy human blood serum [26]. The electrode surface was modified using an electropolymerized poly(3,4-ethylenedioxythiophene) (PEDOT) film which adheres strongly on most electrode materials, shows high conductivity in its oxidized state, presents a good stability in aqueous electrolytes and has biocompatibility with biological media [27]. The voltammetric curves recorded with such a microsensor highlighted an increased UA oxidation current in the presence of AA. This effect was negligible and consequently never previously mentioned in the literature because AA or UA was present in large excess. Comparatively it becomes of great importance in normal physiological conditions as it induces a systematic error in the analysis. The goal of this paper is then to clearly demonstrate the variation of the microsensor sensitivity for UA assay depending on the composition of the sample. A tentative mechanism of UA regeneration by AA at the vicinity of the

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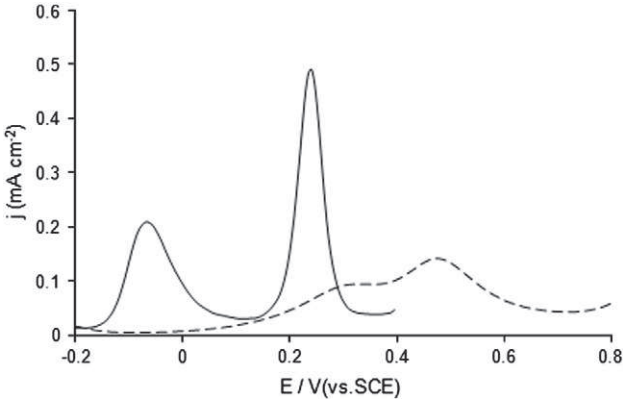

Fig. 1. SWVs recorded with a 50 μm Au microelectrode (dotted line) and the μAu-PEDOT in PBS 0.1 M pH 7.0 containing AA and UA mixtures. Composition of the solutions: AA 0.38 mM and UA 0.30 mM (dotted line); and AA 0.23 mM and UA 0.1 mM (solid line).

modified electrode is proposed based on thermodynamic and kinetic considerations.

2. Experimental

2.1. Chemicals

3,4-ethylenedioxythiophene (EDOT) was purchased from Aldrich. Ascorbic acid (AA) and uric acid (UA) were purchased from Sigma. Tetrabutylammonium perchlorate (TBAPC), potassium dihydrogenophosphate, dipotassium hydrogen phosphate and acetonitrile were purchased from Acros. All reagents were of analytical grade and used as received. The aqueous solutions were prepared with doubled distilled water. High pure nitrogen was used for deaeration.

2.2. Materials

All electrochemical experiments were performed with a Metrohm μ-Autolab potentiostat interfaced to a laptop computer and using the GPES 4.9 software. A three-electrode cell was used for all the experiments. A 50 μm diameter gold wire purchased from Good fellow was used as working electrode. Microdisk electrodes were fabricated by introducing the gold wire in glass capillaries as previously described [28]. A 1 mm diameter platinum wire was used as auxiliary electrode. All potentials reported in the text are referred to a saturated calomel reference electrode (SCE) connected to the cell by a Luggin capillary.

2.3. Preparation of PEDOT-modified microelectrodes

The procedure of PEDOT electrodeposition was similar to that previously reported [26]. Briefly the gold microelectrode was polished with alumina slurry and rinsed with distilled water. The polished surface was then pretreated by cycling the electrode potential between −0.88 V and 1.5 V for 10 min at 10 mV s^{-1} in deaerated acetonitrile containing 0.1 M TBAPC as supporting electrolyte. The polymer was synthesized by cycling the electrode potential between −0.88 V and 1.5 V at a scan rate of 250 mV s^{-1} in the previous electrolytic solution containing EDOT monomer 2.5 mM. The amount of polymer synthesized corresponded to an amount of anodic charge of 12 mC cm^{-2} (about 6.10^{-8} mol cm^{-2}) and was controlled by means of the number of potential cycles during electropolymerization. The modified electrode (hereafter referred as μAu-PEDOT) was finally rinsed with acetonitrile and distilled water to remove any physically adsorbed monomer.

2.4. Electrochemical detection of AA and UA

Experiments were performed with the μAu-PEDOT in 10 mL deaerated phosphate buffer solution (PBS) pH 7.0. The electrochemical detection of AA and UA was based on square wave voltammetry (SWV) in the potential range from −0.2 V to 0.4 V. The potential waveform was optimized with respect to the determination of UA: frequency 25 Hz, step potential 2.5 mV, pulse height 25 mV.

3. Results and discussion

Fig. 1 shows the square wave voltammograms (SWVs) recorded with the unmodified (dotted line) and the μAu-PEDOT modified electrode (solid line) immersed in mixture solutions of AA and UA. Whatever the electrode, no significant signal was observed in PBS blank in the potential range used (result not shown). The oxidation of both acids on the unmodified electrode resulted in two broad, overlapped anodic signals with peak potential E_p close to 0.31 V and 0.48 V for AA and UA respectively. In contrast, two well-defined oxidation signals were recorded with the μAu-PEDOT electrode. The anodic peak potentials for AA and UA were shifted to more negative values, i.e. −0.07 V and 0.24 V respectively, thus demonstrating the catalytic activity of the conductive polymer for the oxidation of both biochemical species. The potential difference was more than 300 mV,

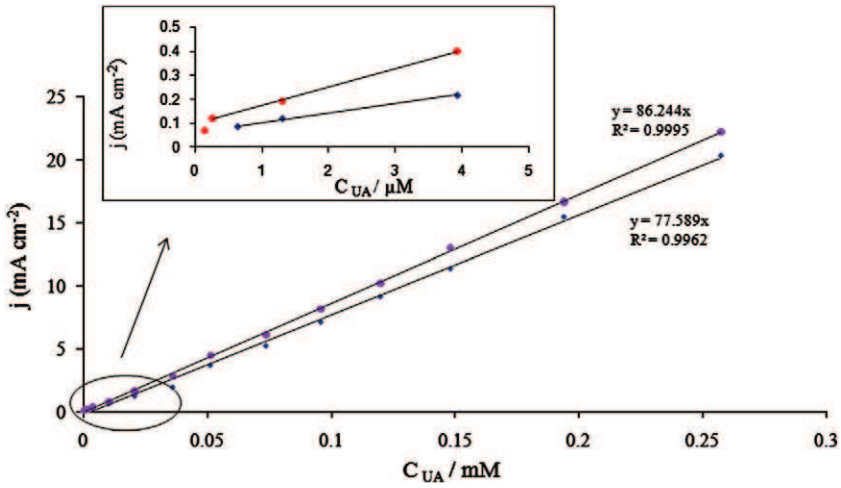

Fig. 2. Calibration curve of the μAu-PEDOT to UA assay without (♦) or with AA 1 mM (●) in the calibrating solutions. Detection potential: 0.24 V. The inset shows the curve for low UA concentrations.

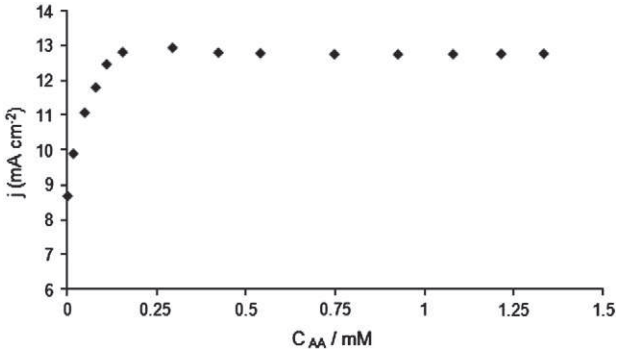

Fig. 3. Influence of the AA concentration on the amperometric response of the μAu-PEDOT to UA 100 μM detection potential: 0.24 V.

allowing the simultaneous detection of both species with the same modified electrode. Furthermore the amperometric responses recorded with the μAu-PEDOT electrode were higher, even with lower concentrations, thus highlighting the improved sensitivity of the resulting microsensor, due to electrostatic and hydrophobic attractions between the polymer and the analytes [29,30].

The analytical performances of the μAu-PEDOT electrode for UA assay were evaluated by plotting the calibration curve (Fig. 2) with or without AA 1 mM in the calibrating solutions. In both cases the peak current density recorded at 0.24 V increased linearly with the concentration for values up to 250 μM with a detection limit of 0.26 μM (S/N = 3). The sensitivity of the microsensor for UA was found to be affected by the presence of AA since it increased from 77.5 mA mM^{-1} cm^{-2} without AA to 86.2 mA mM^{-1} cm^{-2} with AA 1 mM. Actually this variation did not result from an interfering effect of AA due to a poor selectivity since the anodic peaks of both species were sufficiently separated (Fig. 1).

Fig. 3 shows the influence of AA concentration on the amperometric response of the μAu-PEDOT microsensor to UA 100 μM. The sensitivity of the sensor increased gradually for AA concentrations lower than 250 μM and then remained constant for higher values. This current amplification was of course not observed without AA in solution. It was neither due to the polymer since similar results were observed when the experiments were performed on a non-modified microelectrode: by adding AA 150 μM in a UA 300 μM solution, the anodic current at 0.8 V increased about three fold more than that expected by simply adding both anodic signals. This enhanced sensitivity agreed well with an EC' mechanism resulting from a chemical reaction in solution coupled to the electrochemical step [31]. The apparent standard potential of the (ascorbyl radical/ascorbate ion) redox system being lower than that of the (alloxan/urate) one (0.28 V and 0.59 V at pH 7.0 respectively [32,33]), a spontaneous oxidoreduction reaction between AA and the UA oxidation product takes place, thus regenerating the reduced form of UA at the vicinity of the modified electrode surface. In consequence, contrary to previous published papers [12,15], an obvious change was observed in our case in the UA oxidation current depending on whether AA was present in the sample or not. Consequently two different protocols have to be practically adopted depending on the composition of the samples: in the case where all samples contain AA (particularly if in large excess), the assay of UA would induce no bias provided that the calibration curve is performed in the presence of AA. In the case where AA is not present or is in concentration similar to that of UA, AA has to be assayed before UA, the calibration curve of UA taking into account the presence or the absence of AA.

4. Conclusion

Modification of gold microelectrode surface by means of PEDOT electropolymerization has proved to be efficient to elaborate a voltammetric sensor for the simultaneous detection and assay of AA and UA. The sensitivity of the sensor to UA depends sensibly on the concentration of AA in the mixture. This is consistent with an EC' mechanism of UA regeneration by AA at the vicinity of the electrode surface.

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