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Electrochemical detection of uric acid and ascorbic acid: Application in serum

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ABSTRACT

The detection and quantification of uric acid (UA) and ascorbic acid (AA) in different biological fluids such as serum is very important in clinical diagnosis. In this study, a sonogel–carbon electrode modified with L-cysteine was used for detection and quantification of these molecules by cyclic voltammetry (CV) and square wave voltammetry (SWV). These two methods are known for their suitability for monitoring of bioactive molecules in biological samples. Optimization of parameters affecting selectivity and sensitivity of these two methods is included. The peak current showed a linear relationship with the concentrations of UA and AA in the range of 10^{-5} M to 10^{-4} M for UA and 5×10^{-5} M respectively. The detection limit was of 10^{-5} M for UA and 5×10^{-5} M for AA. In addition, the interference of UA and AA was also studied. A neat separation of the oxidation peaks of these two compounds with a peak-to-peak separation of 295 mV was registered. The results indicate that carbon electrode modified with L-cysteine can successfully be used for a selective and sensitive simultaneous determination of UA and AA in the serum.

1. Introduction

The serum uric acid (UA) is the final product of the inert purine metabolism. Excessive synthesis or urinary elimination defect leads to hyperuricemia and increase in serum UA content, which are linked to the occurrence of several diseases such as diabetes, hypertension and gout. It is now established that UA is a powerful reducer of free radicals [1]. For a healthy person the concentration of UA in the serum ranging from about 240 to 520 μmol/L, and into the urinary excretion the concentration ranging from about 1.49–4.46 mmol/L/24 h [2]. The determination of the amount of UA in biological fluids occupies a very prominent place in the diagnosis of various diseases [3]. AA is an essential vitamin in the human diet. It exists in animals and plants, and is known for its antioxidant properties. Also it has been used for the prevention and treatment of the common cold, mental illness, infertility, cancer and some clinical manifestations of human immunodeficiency virus (HIV) [4]. UA and ascorbic acid (AA) are often co-present in human biological fluids, especially in serum, blood and urine. Various colorimetric and enzymatic methods have been widely used to determine the concentration of UA in the presence of AA [5–7], however, these techniques have some gaps and problems such as the complexity, high cost, low sensitivity and selectivity especially in the biological fluid matrix. For a good selectivity and remarkable time gain, electrochemical techniques have received much interest [8–9]. The simultaneous determination of UA and AA by most solid electrodes causes a big problem through the overlapping of signals because the oxidation of UA and AA occurs almost at the same potential. The chemically modified electrodes were recently used to solve this problem for clinical applications [10–13]. The sonogel–carbon modified electrode is a new class of modified electrodes having excellent mechanical and electrical properties, which make them promising for electrochemical determination of different chemical and biological molecules [14–16]. The sonogel–carbon modified electrode by L-cysteine prepared in our laboratory is a special class of sol–gel electrode with outstanding properties as an electrochemical sensor. It has been used for the detection of epinephrine in the presence of UA [17] and for the detection of dopamine [18]. In another line of research, we approached the side bioassay by the development of this electrochemical sensor to detect and quantify electrochemically dopamine in the biological medium (serum) in the presence of potential interference AA and UA [19]. In this context, the sonogel electrodes modified by L-cysteine show a remarkable selectivity and sensitivity, as well as in the buffered medium and in complex biological fluids such as serum. Thus, the influence of several parameters in terms of selectivity and sensitivity was studied and optimized. In this paper, we assayed antioxidant molecules, able to stop or slow the

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oxidative stress, which are UA and AA in biological contexts. We also optimized and controlled the different performances of these sensors in their applications in human serum. Again remarkable results were obtained.

2. Experimental

2.1. Materials and methods

2.1.1. Materials and reagents

L-Cysteine (>99%) was bought from Fluka Chemical Company (Switzerland), uric acid (UA) (99%) was purchased from Sigma (Barcelona, Spain), ascorbic acid (AA) (99%) was obtained from Aldrich (Milwaukee, USA), serum group (A) rhesus negative was provided by blood transfusion center, Tetouan, Maroc, hydrochloric acid (HCl), sulfuric acid, and methyltrimethoxysilane (MTMOS) were from Merck (Darmstadt, Germany), and (H_2SO_4) was from Panreac (Barcelona, Spain). KH_2PO_4 and K_2HPO_4 for phosphate buffer were from Fluka. Graphite powder (spectroscopic grade RBW) was from SGL Carbon (Ringsdorff, Germany). Nanopure water was passing twice distilled water through a Milli-Q system (18 MΩ cm, Millipore, Bedford, MA). Glassy capillary tubes, i.d. 1.15 mm, were used as the bodies for the composite electrodes. All reagents were of analytical grade or higher and used as received without further obtained purification. All experiments were conducted at room temperature with the secondary distilled water.

2.1.2. Apparatus

The voltammetric measurements were performed using a potentiostat/galvanostat type Voltalab PGZ301 (DYNAMIC – EIS VOLTAMMETRY – France). This device was controlled by a computer and processes the chemical data into electrical signals by means of software, which is used also for the acquisition and processing of the results. The experiments were carried out in a three-electrode cell: the counter electrode was a platinum wire and an Ag/AgCl, the 3 M KCl electrode was used as the reference, and the working electrode is the sonogel–carbon/L-cysteine in the form of capillary tubes filled with composite. The techniques used in this work were the cyclic voltammetry (CV) and the square wave voltammetry (SWV). The process of sonocatalysis for the preparation of the electrode sonogel is realized using an electric significator high performance type, MISONIK Inc., Farmingdale, NY, USA. The pH of the solutions was measured and calibrated using a pH meter type PH-METRI GLP22.

2.1.3. Preparation of the sonogel electrode

The 5% L-cysteine Sonogel–Carbon modified electrode was to prepare and test in our laboratory. To prepare the sonosol, the general procedure was as follows: 500 μL of MTMOS and 100 μL of 0.2 M HCl were mixed and then insonated during 5 s with the high-power ultrasonic processor; in this way the mixture is subjected to the phenomenon of ultrasonic cavitation, by which the sol–gel process begins, avoiding the use of alcoholic solvent and reducing drastically the time needed to get a unique phase. In the next step, the adequate amounts of L-cysteine and graphite powder were added and homogeneously dispersed in the obtained sonosol. After several minutes, the resulting material starts to acquire enough consistency, thus it could fill easily the glass capillaries leaving a little extra mixture sticking out of the glass tube to facilitate the subsequent polishing step. After 24 h, the sonogel–carbon L-cysteine composite electrode becomes hardened and, therefore, structured. Adherence between the developed material and the glass was excellent. Before use, the electrodes were polished with No. 1200 emery paper to remove extra composite material and wiped gently with weighing paper. Electrical contact was established by inserting a copper wire into the capillary.

3. Results and discussion

3.1. Electrochemical detection of ascorbic and uric acids

3.1.1. Electrochemical behaviors of AA and UA

The electrochemical behavior of the AA on the carbon sonogel sensor was studied by cyclic voltammetry (CV). Fig. 1 shows cyclic voltammograms obtained on a sonogel–carbon microelectrode in a phosphate buffered solution 0.05 mmol·L^{-1}, pH 7 containing AA 1 mmol·L^{-1}. On the carbon sonogel electrode, an anodic wave corresponding to the oxidation of AA is observed to leave (−50 mV) with a half-wave potential $E_{1/2}$ close to 175 mV. No signal is observed during the scanning back, which means that the system is irreversible. These results are consistent with the literature [20]. The limiting current density measured at 210 mV potential is equal to 177 μA·cm^{-2}. On the sonogel electrode/5% L-cys anodic wave is observed around 210 mV so no oxidation peak displacement is observed relative to the unmodified electrode. The limiting current density measured on the diffusion current is almost three times larger (485 μA·cm^{-2}). Similar experiments were conducted with UA solution 1 mmol·L^{-1}. Fig. 2 shows the cyclic voltammograms obtained. The oxidation of UA on a carbon electrode sonogel is irreversible

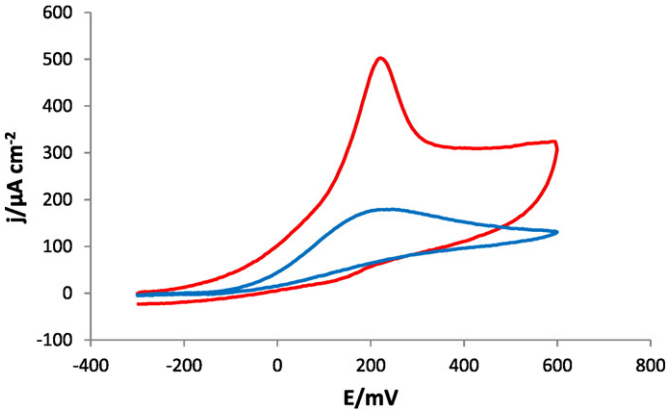

Fig. 1. Cyclic voltammograms obtained in a phosphate buffer solution containing ascorbic acid (pH 7) 1 mmol·L^{-1} on the sonogel–carbon electrode (blue curve) and on the 5% L-cysteine sonogel–carbon modified electrode (red curve). Potential scan rate: 100 mV·s^{-1}.

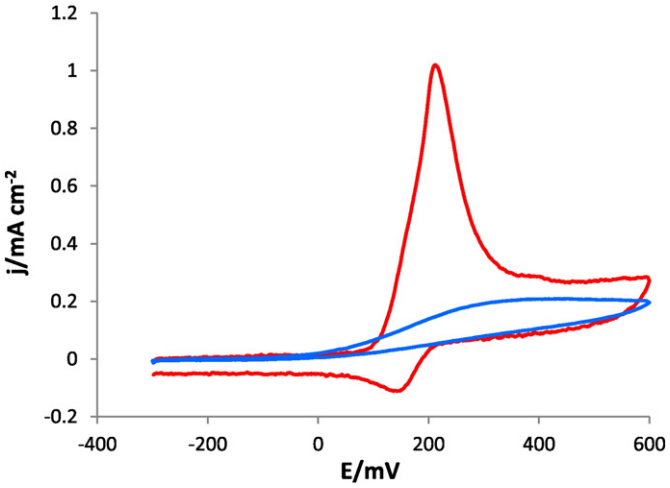

Fig. 2. Cyclic voltammograms obtained in a phosphate buffer solution of (pH 7) containing 10^{-3} M of uric acid on the sonogel–carbon electrode (blue curve) and on the 5% L-cysteine sonogel–carbon modified electrode (red curve). Potential scan rate: 100 mV·s^{-1}.