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Electrochemical determination of α-lipoic acid in human serum at platinum electrode

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ABSTRACT

The voltammetric determination of α-lipoic acid (LA) in human serum was performed at platinum (Pt) electrode in acetate buffer solution. The influence of chloride ions on the electrochemical oxidation of LA was also investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) in buffered aqueous solutions at both Pt and glassy carbon (GCE) electrodes. Pt electrode showed a better electrocatalytic activity related to LA oxidation unlike GCE. The electrochemical oxidation of LA at Pt electrode is a pH-dependent irreversible process involving the transfer of one electron and one proton and also is NaCl-promoted near to neutral pH. The anodic peak current from 0.55 V increases linearly with LA concentrations ranging from 10 to 800 μM at pH 4.5, the limit of detection (LD, determined by using the 3*s*/*b* criterion, where *s* is the standard deviation of the blank signal and *b* is the slope of the calibration plot) being of 13.15 μM. The LA determination in human serum samples was performed by DPV at Pt electrode in acetate buffer solution with no interference from ascorbic acid (AA), dopamine (DA) and uric acid UA). The LA amount in human serum was determined to be 15 μM, with the coefficient of variation of 1.51%.

1. Introduction

During the last period an increasing incidence and prevalence of various chronic diseases such as diabetes, cancer and various neurological disorders has been reported worldwide. Some pathogenic processes were related to oxidative stress induced by the presence of free radicals and heavy metals [1]. Reactive species containing oxygen (ROS) and nitrogen (RNS), the most common free radicals, are released during cellular metabolism acting as soldiers of the human body against viruses and bacteria. When the body cannot quench an excess of free radicals, the health damages occur and various diseases are developed consequently [2]. Various molecules known as antioxidants protect the human body against oxidative stress-induced damages either by free radicals scavenging or by heavy metal ions coordination. The antioxidant are molecules with high molecular weight (HMW) like proteins, enzymes, or low molecular weight (LMW), respectively, such as glutathione (GSSG), ascorbic acid (AA) and uric acid (AA) showing potential health benefits as they restore the balance between redox processes in the human body. One of the most effective LMW antioxidants is α-lipoic acid (LA), a hydro- and liposoluble disulfide, known also as thioctic acid or 1,2 dithiolane-3-pentanoic acid. Notwithstanding LA can be synthesized in the human body from cysteine and fatty acids, the supplementation from exogenous sources becomes very necessary to keep the LA within optimum biological levels and thus to protect the human health against oxidative stress [3]. After supplementation, LA is rapidly converted to its reduced form, dihydrolipoic acid (DHLA) that is involved subsequently in regeneration of other endogenous antioxidants such as GSSG, AA and E vitamin. LA is known also as "universal antioxidant" due to its unique properties such as a very negative redox potential ($E_{LA/DHLAA} = -0.32$ V) [4,5], possibility to cross both the blood–brain barrier and the cell membranes and antioxidant activity displayed both in reduced (DHLA) and oxidized forms. Owing to its valuable antioxidant properties LA is used as preservative in food and cosmetics industries but a considerable attention was given to application of LA in therapy of different diseases or related pathologies since its discovery. Thus, LA was quite hopeful for diabetes and diabetic neuropathy treatment [6–8] and to ameliorate the side effects of the aging [9]. Also, LA proved therapeutic actions for stroke and neurological disorders [10], cancer [11] as well as for glaucoma

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[12]. In addition, LA has therapeutic potential in therapy involving liver pathologies associated with both chemical and mushroom poisoning and with alcoholism as well.

The assaying of LA concentration in biological fluids may be an attractive tool for people health assessment and monitoring if is considered the correlation existing between LA level in biologically fluids and various chronic diseases occurrence [13]. LA determination may improve the accuracy of clinical diagnosis and application of more efficient therapies. Moreover, the determination of LA is useful to quality control testing of pharmaceutical and cosmetic products.

Different chromatographic and spectrophotometric methods have been applied for determination of LA from different biological matrices as well as from dietary supplements. Gas chromatography (GC) [14,15] and high performance liquid chromatography HPLC with fluorimetric [16] or electrochemical detection [17–19] showed good sensitivity for LA determination. UV–VIS spectrophotometry was also employed for LA assaying in pharmaceuticals [20,21].

The electroanalytical methods have attracted a considerable attention in respect to chromatography and spectrophotometry due to their high sensitivity, fastness, simplicity and low costs. In addition, the miniaturization of sensing devices as well as the possibility to discriminate between oxidized and reduced forms open the ways for in vivo monitoring of different biologically active compounds subjected to oxidative reactions in the human body such as thiols [22].

Electroanalytical methods are usually applied in clinical analysis as self-contained techniques or combined with chromatography [23] or spectrometry. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) using a glassy carbon electrode (GCE) have been employed for determination of LA both in pharmaceuticals [24–26] and in human plasma [27]. The sensitivity of the determination was improved on modifying different carbon electrodes by carbon nanotubes [28] and cobalt phthalocyanine [29]. Square wave voltammetry (SWV) on a fluorine-doped tin oxide electrode has been applied for determination of LA from pharmaceuticals [30]. The voltammetric behavior of LA at a mercury electrode was also investigated [31,32]. The coulometric titration with halogens has been employed for LA assaying [33,34] but the reported results were in disagreement. On using the pulsed voltammetric method at a gold electrode the analytical parameters of LA determination were enhanced [35].

The influence of the buffer and NaCl on the electrochemical reduction of LA at mercury electrode was previously reported [31]. In the present work we studied the electrooxidation of LA at an electrode with less toxicity such as Pt and we developed subsequently a voltammetric method for determination of LA in human serum. The novelty of this work lies in the study of the influence of NaCl toward LA oxidation at Pt electrode. The influence of pH on LA voltammetric determination was also investigated. An optimized voltammetric method for determination of LA in human serum was developed subsequently. The analytical performance of the proposed method, in terms of limits of detection and quantification, linear response range and repeatability, was assessed. The results obtained within this study may have a contribution to understanding potential interactions between LA biomolecule and different platinum metal complexes used as chemotherapeutic agents [36,37].

2. Experimental

2.1. Chemicals

All reagents were of analytical grade and have been used without further purifications. *R* (+)(−)-α-LA was purchased from Aldrich. A stock solution of 5 mM LA resulted by dissolving the appropriate amount of LA in a mixture of H_2O: ethanol 1:1 was stored at 4 °C and thereafter used to obtain the final concentrations by dilution with corresponding buffer solutions. The supporting electrolytes used in voltammetric measurements were either 0.1 M phosphate buffer (PB) with pH 6.3, 7 and 8, and 0.2 M acetate buffer with pH of 4.5. Some experiments were conducted in saline phosphate buffer (SPB) containing 0.9% NaCl. Phosphate buffer (PB) solutions were obtained by dissolving appropriate amounts of NaH_2PO_4 and Na_2HPO_4 (Fluka) in doubly distilled water, whereas the acetate buffer solution was obtained from $CH_3COONa{\cdot}3H_2O$ (Sigma–Aldrich) and CH_3COOH.

2.2. Human serum blood samples

Human serum probe was collected from a patient with stroke, stored at −80 °C before measurements and analyzed without any further treatment. The blood sample was diluted (1:24) with 0.2 M acetate buffer pH 4.5 by transferring of 20 μL human serum into 480 μL buffer solution. After that, an appropriate volume of 5 mM LA stock solution was added to the diluted serum sample to achieve a concentration of 100 μM LA. The resulting solution was spiked with 10, 20, 30, 50, 70, 90 and 110 μM LA and subjected to voltammetric measurements in order to obtain the calibration curve.

2.3. Electrochemical measurements

All electrochemical measurements were carried out using an Autolab potentiostat/galvanostat PGSTAT 302N with a GPES 4.9 software and a three-electrode cell at room temperature, in presence of the air. The working electrode (WE) was either a Pt disk of 3 mm diameter or GCE of 2 mm diameter from Metrohm, whilst a platinum wire was used as auxiliary electrode. As pseudo-reference electrode a homemade Ag/AgCl wire calibrated versus $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ was used (E^0 = 180 mV). WE was polished before measurements to mirror finishing using alumina slurries of 1, 0.3 and 0.05 μm and thereafter electrochemical cleaned by CV in 0.5 M H_2SO_4.

The cyclic voltammograms (CVs) were performed within the range −0.2 and 1 V with a scan rate between 10 and 200 mV s^{-1}. The differential pulse voltammograms (DPVs) were recorded within the range from 0 to 1 V in the following experimental conditions: pulse amplitude 50 mV, pulse width 50 ms, step potential 1.5 mV, scan rate of 5 mV s^{-1}. Before starting the measurements, the WE was transferred into buffer solutions and DPVs were recorded until a stable response was obtained in order to obtain reliable electrochemical data. For a precise measurement of the height of the peaks, DPVs were blank subtracted [38].

3. Results and discussion

3.1. Electrochemical oxidation in phosphate buffer solution

Considering the determination of LA in human serum blood samples as main objective of this work, the electrochemical oxidation of LA was studied at 6.3 < pH < 8 in PB solutions, close to physiological pH. Also, taking into account that NaCl is one of the most common electrolytes in the human body, the electrochemical measurements were performed in PB containing 0.9% NaCl.

CV was first employed to study the electrochemical oxidation of LA in SPB solution. The first three consecutive CVs traces for oxidation of 100 μM LA at pH 6.3 in SPB solution are depicted in Fig. 1A. During the first potential scan, an anodic peak is observed