ANALYTICAL SCIENCES AUGUST 2016, VOL. 32

Development of Sensitive Analytical Approach for the Quantification of α-Lipoic Acid Using Boron Doped Diamond Electrode

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A boron doped diamond (BDD) electrode was investigated for use as an electrochemical sensor for α-lipoic acid (LA) using amperometric and differential pulse voltammetric detection. LA displays a well expressed oxidation peak at +0.9 V *vs.* Ag/AgCl in solutions with a pH value of 3. It was found that signals obtained are linearly related to the concentration range from 0.3 to 105 μM with detection limit of 0.088 μM. Interferences by common compounds such as ascorbic acid, uric acid and dopamine were tested and the method was successfully applied to the determination of LA in human body fluids where it gave recoveries in the range from 95 to 97%.

Keywords α-Lipoic acid, BDD electrode, electrochemical sensor, sensitivity

(Received February 10, 2016; Accepted April 21, 2016; Published August 10, 2016)

Introduction

The natural compound α-lipoic acid (1,2-dithiolate-3-pentanoic acid or 6,8-thioctic acid) is synthesized in prokaryotic and eukaryotic cells as a mitochondria product from octanoic acid and cysteine. The uses of this compound have been widely investigated in the treatment of diabetes, various poisoning, glaucoma and cancer cells. α-Lipoic acid in it structure contain disulfide and its use in the treatment of heavy metal poisoning is well known.[1-5] Nowadays, LA is available in a different form for oral intake.[6] Due to all these facts, the determination of LA and monitoring of its concentration in different biological samples was investigated by different research groups. There are already numerous methods reported based on spectrophotometry[7,8] or high performance liquid chromatography[9,10] coupled with different detection systems due to their advantages on sensitivity and selectivity. Up to date, there are also several methods reported in the literature, dealing with the determination of LA using different electrodes, such as fluorine doped tin oxide,[6] platinum,[11] mercuric lipoate ion-pair,[12] hanging mercury drop electrode,[13] highly oriented pyrolytic graphite (HOPG) and glassy carbon electrode,[14] and employing different detection techniques. Determination of lipoic acid is also proposed by Siangproh[15] *et al.* using boron doped diamond electrode and HPLC system. This electrode is proposed as a powerful sensor for HPLC system by Mahe[16] *et al.* These methods possess several disadvantages and drawbacks such as, high cost and complexity of instruments, time consuming sample preparation and long analysis time. For some of these techniques presence of interfering compounds can be a limit in a factor for real sample application. Due to these facts, attempts to improve these methods led to the development of a simple, fast and reliable electroanalytical approach for LA quantification.

Electroanalytical methods such as amperometry, differential pulse voltammetry or square wave voltammetry in combination with appropriate electrode material could offer characteristics to pass all above mentioned disadvantages. Boron doped diamond electrode is nowadays one of the best solid electrode materials, with low background current, negligible surface passivation, wide potential range and it was used for quantification of many biologically active compounds in the field of environment, food and drug analysis.[17-19]

The aim of this study was to investigate application of BDD electrode for the determination of biological important compounds based on different detection techniques. Influence of most common interfering compounds that could be found in human body fluids was investigated. After optimization of all the experimental parameters that can strongly affect on the characteristics of one electroanalytical procedure, proposed method was successfully applied for LA quantification in human urine samples, with satisfactory recovery.

Experimental

Reagents

All reagents used in this study were of analytical grade. Solutions were prepared in ultrapure Milli-Q water (Millipore system). Britton–Robinson buffer solution (BRBS) was prepared by mixing phosphoric acid, boric acid and acetic acid, all in the concentration of 0.04 M.

Equipment

The voltammetric measurements were performed using a potentiostat/galvanostat (Autolab PGSTAT 302 N, MetrohmAutolab B.V., The Netherlands) controlled by the corresponding electrochemical software (NOVA 1.10). The electrochemical cell with the total volume of 15 mL was

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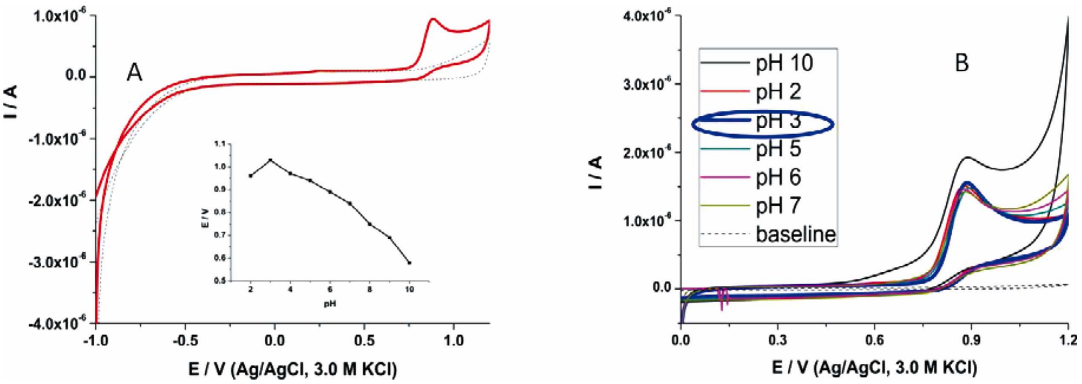

Fig. 1 (A) Electrochemical behavior of 0.1 mM LA at BDD electrode in BRBS at pH 3; inset figure presents dependence of peak current from pH. (B) Cyclic voltammograms of LA oxidation at BDD electrode at various pHs of BRBS.

equipped with a boron-doped diamond electrode (i.d., 3 mm; Windsor Scientific Ltd., UK) embedded in a polyether ether ketone (PEEK) body with an inner diameter of 3 mm, a resistivity of 0.075 Ω cm and a boron doping level of 1000 ppm (as declared by the supplier) was used as the working electrode, an Ag/AgCl (3.0 M KCl) (potentials reported in this paper are referred *versus* this electrode) as a reference electrode and a Pt wire as a counter electrode. All measurements were done at an ambient temperature. pH values of BRBS were adjusted with 0.5 M sodium hydroxide using Orion pH meter (Model 1230) and combined glass electrode.

Analytical procedure

Electrochemical behavior of the α-lipoic acid was investigated using cyclic voltammetry. The potential was swept from 0.0 to +1.0 V and −1.0 to +1.2 V, at the scan rate of 50 mV/s (if not stated otherwise). Selection of the appropriate pH of the supporting electrolyte and investigation of the nature of electrochemical reaction on the BDD electrode surface was done from these measurements. For analytical application, differential pulse voltammetry (DPV) and amperometry were selected and in order to provide best analytical response different experimental conditions were optimized. Recovery measurements were done by adding a known amount of LA standard solution and comparison of obtained results and calculated amount. The potential for DPV measurements was also swept from 0.0 to +1.2 V. The amperometric measurements were done at a potential of +0.9 V. The limits of detection (LOD), for both procedures, were calculated using the equation:

$$\mathrm{LOD} = 3 \times \sigma_{\mathrm{intercept}}/\mathrm{slope}.$$

Sample preparation

The urine samples were collected from healthy and non-smoking volunteers and for the measurements of LA concentration, each 1 mL of fresh urine sample was taken and diluted to 10 mL with Britton–Robinson buffer at pH 3 and then directly analyzed. Before giving the sample, the volunteers were asked not to take any medication. Samples were spiked with aliquots of stock solution of LA before diluting, to provide an increase in LA concentration to the value of 1 µM and make it measurable.

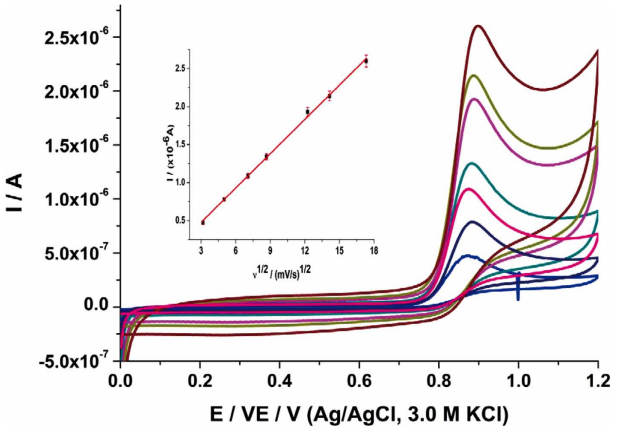

Fig. 2 Cyclic voltammograms obtained for oxidation of 0.1 mM of LA at BDD electrode in BRBS at pH 3 and scan rates from 10 to 300 mV/s. Inset figure present linear dependence of oxidation peak current from the square root of scan rate.

Results and Discussion

Electrochemical behavior of LA at BDD electrode, influence of pH and scan rate

Electrochemistry of α-lipoic acid was studied at the BDD electrode using cyclic voltammetry. Recently reported data indicate oxidation of LA as an irreversible process using platinum, glassy carbon and some different modified electrodes.[11–14] We studied its electrochemical behavior in the potential range from −1.0 to +1.2 V (Fig. 1A). In this potential window, LA provides oval-shape and well-defined oxidation peak at a potential of around +0.9 V. In the reverse scan, no reduction peak was observed indicating that the oxidation process of LA at the BDD electrode in BRBS at pH 3 is irreversible process, which is in accordance with previous studies from other authors. Effect of pH on peak potential and peak current was studied in pH range from 2 to 10 at BDD electrode. It was found that increase of pH causes negligible shifts in peak potential. The obtained current has its highest value at pH 3, where background current was lowest and taking account of peak shape together with current value, for further experiments pH 3 was selected as optimum. Cyclic voltammograms

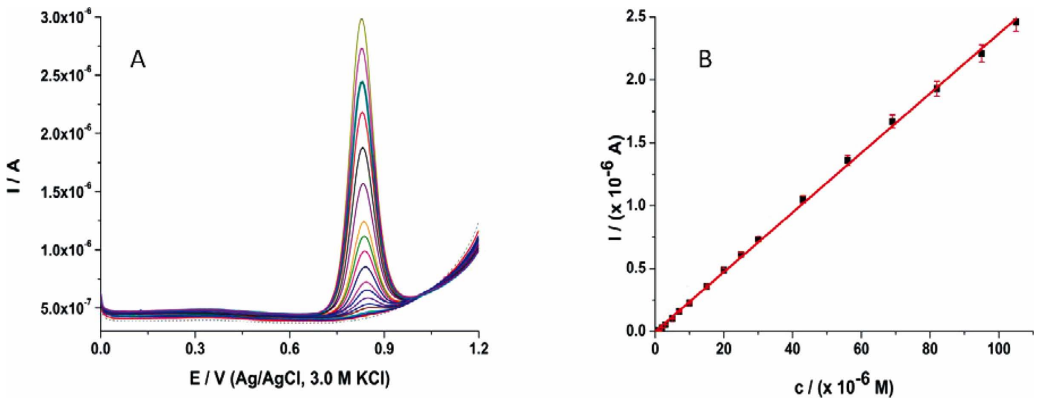

Fig. 3 (A) DPV voltammograms for LA quantification under optimized conditions in the concentration range from 0.3 to 105 µM. (B) Resulting calibration curve obtained from these measurements.

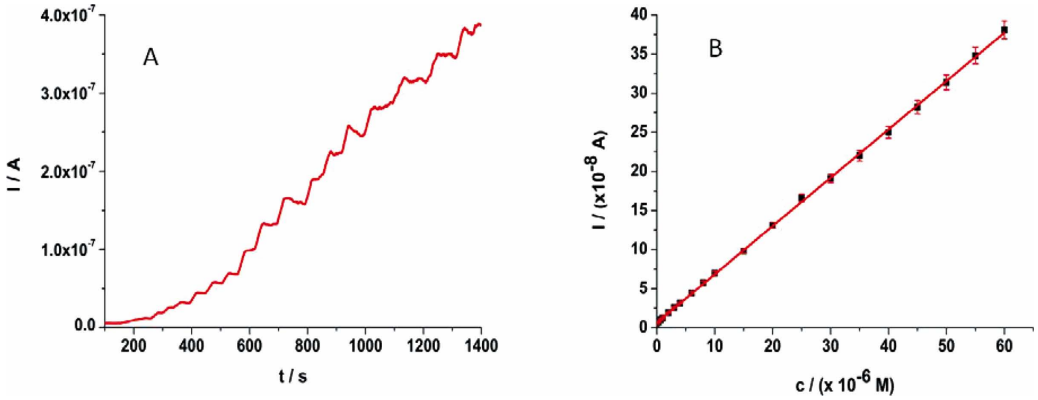

Fig. 4 (A) Amperogram obtained for quantification of LA at BDD electrode at potential of +0.9 V in BRBS at pH 3. (B) Corresponding calibration curve obtained in the concentration range from 0.3 to 65 µM from these measurements.

for oxidation of LA at different pH are depicted in Fig. 1B. These results are in accordance with previous studies and could confirm the hypothesis that electrochemical reaction is oxidation of sulfide leading to formation of different lipoic acids S-oxides, which has been previously reported that belongs to one electron charge transfer and irreversible processes and is pH independent.[14,20]

At different scan rates, the oxidation current increased linearly with the increasing square root of the scan rate (Fig. 2). The corresponding linear equation for these measurements is $I(\mu A) = 0.150v^{1/2}(mV/s)^{1/2} + 0.024$ with regression coefficient $R^2 = 0.9980$. The obtained value indicates that the process at the BDD electrode surface is controlled with diffusion, rather than adsorption.

Analytical performances

Differential pulse voltammetry is a widely used electroanalytical technique for quantification of biological active compounds.[17-19] Low background current, high sensitivity and selectivity are advantages that this method offers. Before construction of a calibration curve some parameters which could strongly improve these characteristics, such as pulse amplitude, pulse time and scan rate, were optimized. While one parameter was optimized, others were kept fixed. The potential was scanned from 0.0 to +1.2 V. Pulse amplitude was varied in the range from 10 to 100 mV. The highest current, taking into account also peak shape, was observed at a value of 70 mV. In the case of pulse time, a decrease in the peak current was observed immediately after starting further increase from 10 ms. At the scan rate of 25 mV/s the best peak characteristics were observed. Based on these facts, pulse amplitude 70 mV, pulse time 10 ms and scan rate of 25 mV/s are selected as optimum and used for all further experiments.

Construction of calibration plot

A calibration curve for DPV measurements was constructed by plotting oxidation peak current *versus* LA concentration, recorded at optimized experimental parameters (Fig. 3A). The proposed electrode showed a wide linear range for LA quantification in the concentration range from 0.3 to 105 µM with a detection limit of 0.088 µM. Corresponding linear equation was found as $I(\mu A) = 0.0236c(\mu M) - 0.001$ with regression coefficient 0.9992. The calibration curve is depicted in Fig. 3B.

Amperometric detection could offer fast and continuous measurements and also present a powerful tool for analysis of biological active compounds. As the LA nowadays is often used for oral intake in different pharmaceutical forms, capsules or tablets, for the treatment of many diseases, this technique could be a successful analytical method for its quantification. For that purpose, analytical response of BDD electrode was investigated toward amperometric detection of LA. The measurements were done at a potential of +0.9 V, selected from previous studies, and results are depicted in Fig. 4A.

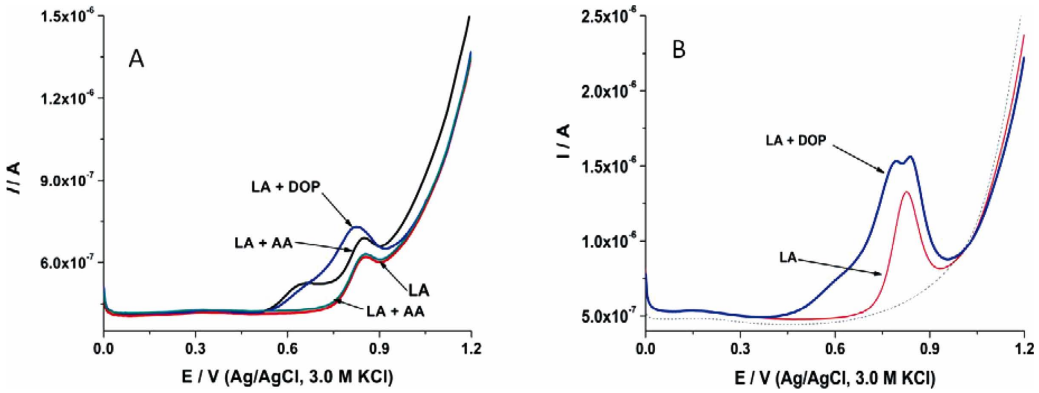

Fig. 5 (A) DPV voltammograms obtained for 5 µM of LA in the presence of dopamine (DOP), ascorbic acid (AA) and uric acid (UA) at the same concentration value, under optimized experimental conditions. (B) DPV voltammogram of 5 µM of LA and dopamine at pH 6 of BRBS.

Addition of certain amount of the standard LA solution was done every 50 – 60 s and the currents for calibration curve were taken at the middle of that period. It was found that this electrode under these conditions possess linear concentration range from 0.3 to 60 µM (Fig. 4B) with corresponding equation $I(\mu A) = 0.618c(\mu M) + 0.665$ where R^2 was found as 0.9997. The LOD for this technique was calculated to be 0.06 µM.

The repeatability of 10 measurements of 0.5, 2 and 10 µM of LA with both proposed methods was around values of 3.1, 2.2 and 1.9%, respectively, which confirms all advantages of the BDD electrode. Obtained values for LOD for both techniques were lower or comparable with those proposed in the literature either for electroanalytical methods or other analytical procedures. A lower detection limit for 0.012 µM with the electroanalytical method was obtained by Alarfaj[13] using a mercury electrode, but nowadays, this electrode is avoided due to toxicity of mercury. LODs proposed by Abbas[12] *et al.* (0.09 µM), Miranda[6] *et al.* (3.68 µM) and Marin[11] *et al.* (13.15 µM) were higher than is our study, which confirm all the advantages of our procedure and working with a BDD electrode.

Interferences studies

Practical application of analytical procedure is usually limited with its selectivity. Possible interfering compounds could possess similar characteristics as target analyte and cause impossible determination in some matrixes. In pharmaceutical formulation, tablets and capsules, along with LA are microcrystalline cellulose and gelatin. They usually contain 300 mg of LA (http://www.puritan.com/alpha-lipoic-acid-530). As these two accompanies are not electrochemically active amperometric determination proposed in this study could be a powerful method for fast and simple LA quantification in these samples. However, in the case of samples such as human body fluids, which could contain ascorbic acid, uric acid and dopamine, their effect on LA quantification under proposed condition, was investigated. Figure 5A shows electrochemical response of 5 µM of LA in the presence of above mentioned compounds at the same concentration level. The peak currents were calculated in the presence and absence of possible interferences. It was considered that tested interferences strongly influenced analyte determination if peak current changes more than 10%. From these measurements it can be concluded that the presence of ascorbic acid and uric acid at the same level as LA does not provide signal changes of LA oxidation. On the other hand, the presence of dopamine at the same concentration ratio with LA causes peak current increases, due to it similar oxidation potential. It is well known that dopamine oxidation is pH dependent and according our results (Fig. 1B) and results from other authors where the LA oxidation process is pH independent, it could be expected that increasing the supporting electrolyte pH can split the oxidation peaks of these two compounds. DP voltammograms at pH 6 of BRBS in the presence of both compounds are depicted in Fig. 5B. It is obvious that an increase of pH to the value of 6 does not split a oxidation signals enough to allow LA quantification in the presence of a high concentration of dopamine. Further increase of pH causes an increase in background current (see Fig. 1B, pH 10) and lowering LOD to the values of 1 µM. Based on these facts, tolerance level was calculated for dopamine at pH 3. It was found that LA can be easily determined, under proposed conditions in the presence of dopamine up to 20 concentration percentage. The higher presence of dopamine in urine samples of the people who had ingested LA tablets or capsules is not expected, and this implies that the proposed method can be used for LA quantification in urine samples with the proposed procedure.

Table 1 Results (µM) obtained for LA quantification and recovery[a] experiments using proposed DPV method

Sample	Found	Added/ found	Recovery, %	Added/ found	Recovery, %
1	1.02	2.00/3.03	101	2.00/5.10	102
2	1.03	2.00/3.03	100	2.00/5.12	102

a. Recovery was calculated as: [LA]found/[LA]added × 100%.

Practical application

The proposed procedure was applied for quantification of LA concentration in spiked urine samples. These samples were prepared as it is mentioned in the Experimental section. The results are summarized in Table 1. All results listed in Table 1 are estimated with the DPV method, calculated from the calibration curve and present mean value of three measurements. In order to evaluate matrix effect, recovery experiments were done. It can be concluded that obtained values are in good agreement with prepared artificial samples. Recovery measurements were in the range from 100 to 102%, indicating that the proposed method can be successfully used for

quantification of LA in real samples and can be a satisfactory replacement for time-consuming and expensive separation methods.

Conclusions

In this study sensitive and selective methods for quantification of α-lipoic acid with one of the best solid electrode materials are proposed. This determination is based on electroanalytical techniques, amperometry and differential voltammetry. Both optimized procedures possess wide linear working range with one of the lowest LOD. Practical application of the DPV method is shown in LA analysis in spiked urine samples, and obtained results together with results from recovery experiments were satisfactory, indicating that these methods could be promising analytical approaches for successful quantification of this analyte.

Acknowledgements

This work was supported by the Ministry of Education and Science of the Republic of Serbia (project No. OI172030) and JoinEU-SEE-Penta Erasmus Mundus scholarship.

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