Anodic Oxidation of α-Lipoic Acid on Carbon Electrodes in Acetic Acid – Acetonitrile Solutions

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α-Lipoic acid (LA) is a powerful natural antioxidant with anti-inflammatory properties and anti-cancer effects. The aim of this work was to study the electrochemical properties of LA in acetic acid solutions in order to develop a new voltammetric method for its determination. Voltammetric behavior of LA on both a carbon fiber microelectrode (CF) and a glassy carbon macroelectrode (GCE) in an optimized composition of the solutions of acetic acid with acetonitrile (20%, *v/v*), as well as sodium acetate as a supporting electrolyte, was investigated. The voltammetric techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and linear sweep voltammetry (LSV) were used for this purpose. The voltammograms recorded on the carbon electrodes show a well-shaped single wave or peak at the potential of above 0.96 V vs. Ag/AgCl. Lack of the cathodic peak on CV curves indicates that the product of the electrode reaction is unstable and undergoes a subsequent chemical reaction. The electrode process of LA was characterized as quasi-reversible, diffusion-controlled, and proceeding with the exchange of one electron. Its products are chemically unstable and participate in irreversible homogeneous reactions resulting in non-electroactive products (E_qC_i mechanism).

1. INTRODUCTION

Proper functioning of living organisms requires the presence of many important compounds. The processes that occur with their participation ensure energy production, in addition to being responsible for growth, protective mechanisms, and contact with the environment. Living cells also contain substances whose presence sometimes causes their damage. *Reactive Oxygen Species* (*ROS*) are among them. Their presence at the physiological level protects organisms against pathogens, and thus ensures inner homeostasis. The increased concentration of *ROS*, which causes the disruption of equilibrium, is a result of stress, poor diet, or lack of physical activity. Their excess reacts with the major components of

the organism: proteins, lipids and DNA. These adverse processes may cause damage to their structure and lead to dangerous diseases caused by oxidative stress [1]. They may also accelerate the body aging process [2]. Antioxidants, as natural allies, protect the body against the harmful effects of *ROS*. Their sources are plant and animal products in a daily diet. This group of substances includes, for example, ascorbic acid (vitamin C), tocopherol (vitamin E), vitamin D, coenzyme Q_{10}, and also α-lipoic acid (LA). Strong antioxidant properties of LA cause that this compound is called as "*universal antioxidant*" [3,4] and "*antioxidant of antioxidants*" [5,6].

Initially, α-lipoic acid (LA; thiooctanoic acid; 6,8-dithooctane; 1,2-dithiol-3-pentanoic acid; 1,2-dithiol-3-valeric acid, Fig. 1) was considered to be a vitamin, but detailed studies have shown that its small amounts can be synthesized from octanoic acid and cysteine by plant, animal and human cells [5,6]. LA is soluble both in an aqueous and fatty environment and therefore protects both lipid membranes and intercellular spaces [7].
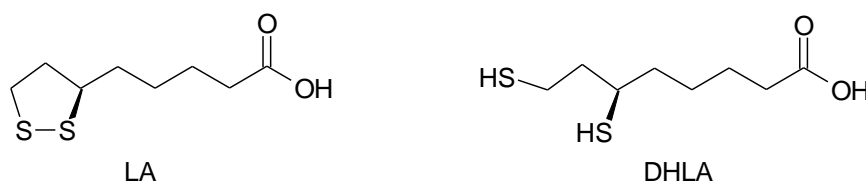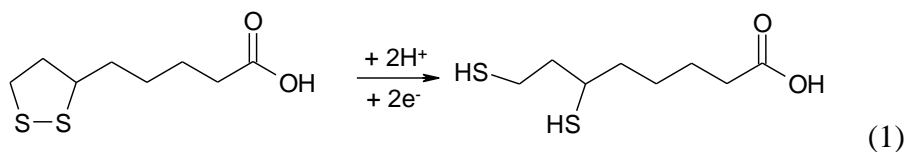

Figure 1. Molecular structure of α-lipoic (LA) and dihydrolipoic acid (DHLA).

The small size of the molecule allows fast penetration of LA into the cells, where it is transformed by reducing the dithiol ring to dihyhrolipoic acid (DHLA):

$$(1)$$

Both LA and DHLA have antioxidant properties and act as scavengers of free radicals [2,8]. Due to the low negative redox potential ($E^{0'}= -0.29$ V [9] or -0.32 V [4]), DHLA is a strong reducer. In addition, it regenerates vitamins C, E, and other important antioxidants, such as coenzyme Q_{10}, glutathione from their oxidized forms, and thus it prolongs and strengthens their action. The characteristic feature of LA is the possibility of both oxidation and reduction. These processes take place on the sulfur atom of the dithiol ring. It is a unique property in comparison to other biologically active substances [3]. However, the antioxidant properties of LA are weaker than its reduced form. Both LA and DHLA form chelate complexes with redox-active metals and thus also prevent the oxidation processes initiated by them [1,7,10]. LA is used in medicine, among others, for therapy of different diseases: cardiovascular [11], multiple sclerosis [8] Alzheimer's disease [12], type II diabetes, diabetic neuropathy [5,7], obesity [13], liver pathologies associated with alcoholism, heavy metals and mushroom poisoning [9,14]. It can also alleviate the consequences of anticancer treatment [6,7], AIDS and HIV [7,15].

Experiments on animals have shown that the use of LA does not cause adverse effects. The estimated LD_{50} of the experimental animals is 400-500 mg per kg body weight. In diabetics, hypoglycemia may occur at high LA doses resulting in increased glucose consumption. There is no data on the carcinogenic and teratogenic effects of LA. Despite the lack of adverse effects, it is not recommended for pregnant women [16].

The natural form of LA is covalently bound to the ε-amino group in the lysine residues to form lipoyllysine. The concentration of LA in the blood serum is 1-25 ng mL^{-1} [6]. However, such an amount does not ensure proper functioning of organisms. Therefore, LA should be supplied from external sources. A very rich source of lipoyllysine is spinach, broccoli, tomatoes, garden peas, Brussel sprouts, as well as red meat and animal-derived foods (kidney, heart, liver and others) [6]. LA amounts delivered to the body only in the form of natural ingredients ensure the proper course of metabolic processes, but are insufficient for therapeutic purposes. Therefore, its deficiencies can be supplemented.

The widespread presence and universal therapeutic use of LA cause that it is necessary to know its properties, including electrochemical ones that are directly related to redox reactions in living organisms. According to literature data, the electrochemical properties of LA were not been frequently investigated. Electrochemical research studies focused mainly on the oxidation of LA, and less frequently on its reduction [3,17,18]. They were often a preliminary step in optimizing conditions for LA determination by voltammetric techniques, or for testing electrochemical detectors used in liquid chromatography (LC). They were mainly carried out in aqueous buffered solutions with different pH: phosphate [14,19-22], Britton-Robinson [23,24], and rarely acetate [19,25]. Limited solubility of LA in water [26], causes that this analyte is initially dissolved in the water-ethanol mixture (1:1, *v/v*) [14,19,25], and then diluted with the appropriate buffer. The most commonly used material for voltammetric investigations of α-lipoic acid has been mainly glassy carbon (GCE) [14,19,21], and platinum [14], gold [17] or mercury [3]. For this purpose, modified electrodes: boron doped diamond (BDDE) [22,23], fluorine-doped tin oxide (FTO) [24], pyrolytic graphite modified with cobalt phthalocyanine (PG/CoPc) [20] or manganese(VI) oxide-modified screen-printed graphene (MnO$_2$/SPGE) [25] were also used. The research was carried out with cyclic (CV) [14,17-25], differential pulse (DPV) [14,19-21,23] and square wave (SWV) voltammetry [19,24,25].

The analysis of the literature data show that the LA oxidation reaction in aqueous buffer solutions occurs in one step, is irreversible, and the electrode process is diffusion-controlled [19,20,23,24]. There is a disagreement among authors on the number of electrons transferred in this reaction. Some of them claim that oxidation of LA is one-electron process [14,19,23] and occurs without [19,23] or with the participation of protons [14]. Corduneanu *et al.* [19] investigated the oxidation of LA on the GCE electrode in a phosphate buffer at pH 6.9. The cyclic voltammograms showed one anodic peak at the potential of 0.8 V vs. Ag/AgCl. However, the cathodic peak was not observed. This means that the one-electron oxidation products of LA are not electroactive, and the electrode reaction is pH independent. The authors suggest that the process proceeds on one of the sulfur atoms, which gives thiosulfinate (S-oxide, β-LA) as a product. They also observed that the use of an acetate buffer (pH 4.1) promoted the adsorption of LA on the surface of GCE. The study of the LA oxidation on GCE and BDDE was also carried out as a preliminary step to their determination by LC [22]. A mixture of an acetonitrile and phosphate buffer pH 2.5 (1:1, *v/v*) was applied. The authors found that the electrode material did not

influence the LA oxidation peak potential. The higher sensitivity of determinations was observed on BDDE.

Marin *et al.* [14] observed that the addition of NaCl to the phosphate buffer makes the oxidation of LA on Pt electrode an irreversible process controlled by adsorption. The anodic peak observed on the CV curves at 0.74 V vs. Ag/AgCl corresponded to the transfer of one electron and one proton giving β-LA as the final product.

In other available works [20,21,24], it is stated that two electrons are involved in the oxidation of LA in an aqueous medium with the formation of thiolsulfinate as a product. It was confirmed by Miranda *et al.* [24] with the use of the SWV technique on the FTO electrode in H_2SO_4 solution (pH 2). Under such conditions, a well-shaped LA oxidation peak was observed at the potential 0.95 V vs. Ag/AgCl. This electro-oxidation process occurs *via* the E_qC_i mechanism, where the water molecule attacks the product of electron exchange reaction with the formation of thiolsulfinate as the final product. It can be described by a general equation:

$$(2)$$
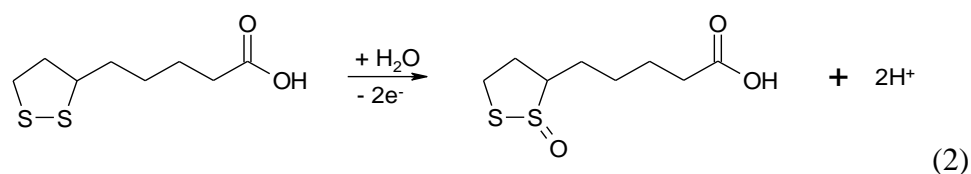
According to the literature review, organic solvents were not used to study LA oxidation. The exception is the use of acetonitrile with tetraethylammonium perchlorate (TEAP) as a supporting electrolyte [17]. A single oxidation peak, corresponding to the quasi-reversible exchange of one electron on the gold electrode, was observed at 1.17 V vs. SCE. A relatively stable radical of α-lipoic acid is the product of the electrode reaction. This is evidenced by the existence of a small signal on the cathodic scan.

So far, there has been no literature data on the electrochemical properties of LA in acidic organic solvents, for example in acetic acid. The aim of our research was to investigate the electrochemical properties of LA in this solvent. The analyte is well soluble in this medium, which also allows the dissolution of the hydrophilic supporting electrolyte necessary for the study of electrochemical processes, as well as the hydrophobic matrix present in preparations containing LA. This may be important during the development of a voltammetric method for their determination in real samples. The significant advantage of acetic acid is a wide range of anodic stability [27]. Additionally, this solvent is safe for human and the environment.

2. EXPERIMENTAL

2.1. Chemicals and reagents

The following reagents were used in the tests: (±)-α-lipoic acid (LA, ≥ 99%, Sigma-Aldrich), sodium acetate (AcNa, anhydrous > 99.0%, Fluka), sodium perchlorate ($NaClO_4$, anhydrous > 98.0%,

Sigma-Aldrich), acetic acid (AcH, p.a. ACS, Merck), acetonitrile (AN, p.a. ACS > 99.5%, Merck), ethanol (EtOH, ACS, Ph Eur, Merck).

2.2 Instrumentation

The studies on the electrochemical properties of α-lipoic acid were carried out using an M161 digital electrochemical analyzer and an M162 pre-amplifier for measuring small currents, cooperating with the EALab 2.1 and mEALab 2.1 software (mtm-anko, Cracow). A three-electrode cell was used, in which working electrodes were microelectrodes: carbon fiber (CF, $\Phi = 33$ μm), platinum (Pt, $\Phi = 10$, 20, 25, 50 μm) or a glassy carbon macroelectrode (GCE, $\Phi = 3$ mm, $A = 0.283$ cm^2). The use of small electrodes was intended to reduce the ohmic potential drops, *IR* between the electrodes in the organic solvent of low dielectric constant (acetic acid, $\varepsilon = 6.19$ [28]). The potential was measured and reported against an external silver chloride reference electrode with 1 M NaCl solution, Ag/AgCl. This electrode was isolated from the test solutions by a salt bridge with a frit of Vicor Glass. The platinum wire was used as an auxiliary electrode. Before each measurement, the surface of the working electrodes was polished on a cloth with 0.05 μm alumina powder, rinsed with deionized water and dried. In order to limit the influence of electrical interferences on the measured signals, the electrolytic cell was placed in the Faraday cage.

3. RESULTS AND DISCUSSION

An important stage of electrochemical research is the choice of optimal composition of a solution. Since LA exhibits limited solubility in water (0.127 g L^{-1} [26]), a suitable reaction environment was sought among organic solvents, such as ethanol, acetonitrile and acetic acid. When choosing a solvent, it should also take into account the possibility of dissolving hydrophobic components accompanying the analyte in real formulations. This is important during the voltammetric determination of LA. The most commonly used electrode material for electrochemical studies of LA was carbon [14,19,21]. Therefore, a carbon fiber microelectrode (CF, $\Phi = 33$ μm) was used for the preliminary tests. At this stage of the experiments, differential pulse voltammetry (DPV) was used. This measuring technique ensures the reproducibility of successively recorded curves by limiting the contribution of adsorption phenomena accompanying the oxidation process. Additionally, it a good resolution of signals can be obtain. DPVs were recorded using optimized parameters: the pulse amplitude of 20 mV, the pulse width of 80 ms, and the scan rate of 20 mV s^{-1}.

The tests started with the use of a mixture of ethanol and water (1:1, *v/v*), containing NaClO$_4$ or AcNa (both at the concentration of 0.1 M) as a supporting electrolyte. DPV curves (Fig. 2 (*a*, *b*)) give the LA oxidation peak at 0.910 V and 0.895 V vs. Ag/AgCl, in the presence of NaClO$_4$ and AcNa, respectively. Their broad peak with a small current, however, indicates that the electrode process is irreversible. This conclusion is confirmed by the literature data on aqueous solutions [14,19,20,23,24]. The feature that distinguishes the curves recorded in the presence of NaClO$_4$ is the significantly higher peak currents. The probable reason for this is the adsorption of acetate ions on the surface of the

electrode, which limits the availability of LA molecules and the exchange of electrons is difficult. The same effect can be observed in ethanol solutions (Fig. 2 (*c, d*)). Additionally, the shift of the LA oxidation peak towards higher potential (1.025 V and 0.985 V in the presence of AcNa and NaClO$_4$, respectively) was observed. The character of the process was still irreversible. Moreover, the curves recorded both in the ethanol and the ethanol-water mixture were not reproducible. In search of the reproducibility of the results, acetonitrile, a popular solvent in electrochemical research, was used. Due to the insolubility of AcNa in this solvent, only NaClO$_4$ was used as a supporting electrolyte. A typical DPV curve of the LA oxidation in acetonitrile is shown in Fig. 2, (curve *i*). A well-shaped signal was obtained at 0.920 V. The process of the exchange of electrons is similar to a reversible one, and the peak current is much larger than the previous ones. This is due to the lower viscosity of acetonitrile compared to ethanol, and water ($\eta_{AN} = 0.341$ mPa s, $\eta_{EtOH} = 1.083$ mPa s, $\eta_{H2O} = 0.890$ mPa s [28]). Consequently, mass transport to the electrode surface increases. Unfortunately, the successively recorded curves were not reproducible in this solvent, either.
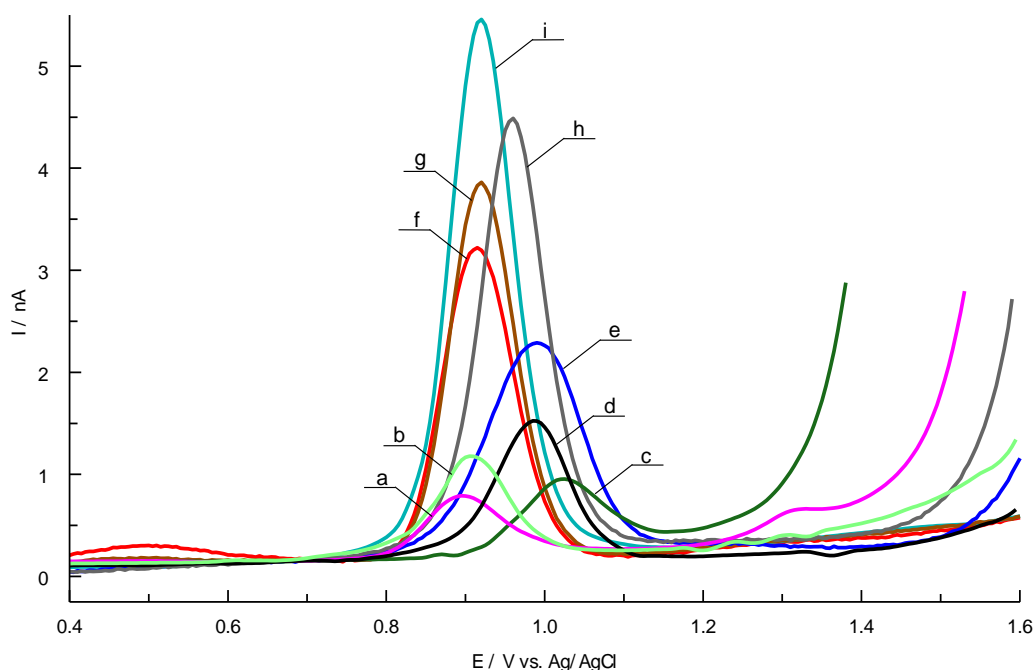

Figure 2. DPVs of LA oxidation (0.5 mM) recorded on CF in: (a, b) mixture of EtOH and H$_2$O (1:1, *v/v*), (c, d) EtOH, (e, f) AcH, (g, h) mixture of AcH and AN (20%, *v/v*), (i) AN. Supporting electrolytes were (a, c, e, h) 0.1 M AcNa, and (b, d, f, g, i) 0.1 M NaClO$_4$.

In the next stage of the study, acetic acid was used. Due to its higher viscosity ($\eta_{AcH} = 1.130$ mPa s [28]), the observed peak current decreased (Fig. 2 (*e, f*)). In the solutions containing sodium perchlorate, the electrode process is more reversible, and the peak current is higher than in the case of AcNa. The reason for this, similarly to the ethanol and the ethanol-water mixtures, is probably the adsorption of acetate ions on the surface of the working electrode. The oxidation of LA occurs at a higher potential ($E_p = 0.990$ V), which may indicate that the process proceeds on a partially blocked electrode. The reproducibility of the curves was still unsatisfactory.

The best results were obtained in acetic acid containing 20% acetonitrile (*v/v*). The presence of AN ($\varepsilon = 35.9$ [28]) increased the electric conductivity of the solutions and facilitated the charge transfer between the electrodes. As a result, a well-shaped peak was observed on the DPV curves recorded both in the presence of NaClO$_4$ and AcNa (Fig. 2 (*g, h*)). Additionally, an increase in peak currents and an excellent reproducibility of successively recorded curves was observed. A higher peak current observed in solutions containing AcNa may result in an increase in the sensitivity of the future determinations. A slightly higher LA oxidation potential in the presence of AcNa compared to NaClO$_4$ (the peak potentials, E_p 0.960 V and 0.920 V, respectively, Table 1.) should not affect their results.

Among all the tested solutions, the mixture of acetic acid and acetonitrile (20%, *v/v*) containing sodium acetate as a supporting electrolyte was selected. This solution composition can be considered optimal and will be applied to the investigations of the electrochemical properties of LA.

3.1 Anodic Oxidation of LA

3.1.1 Voltammetry on Microelectrodes

The study of the electrochemical properties of α-lipoic acid started with a linear sweep voltammetry technique (LSV) on a carbon fiber (CF) disk microelectrode.
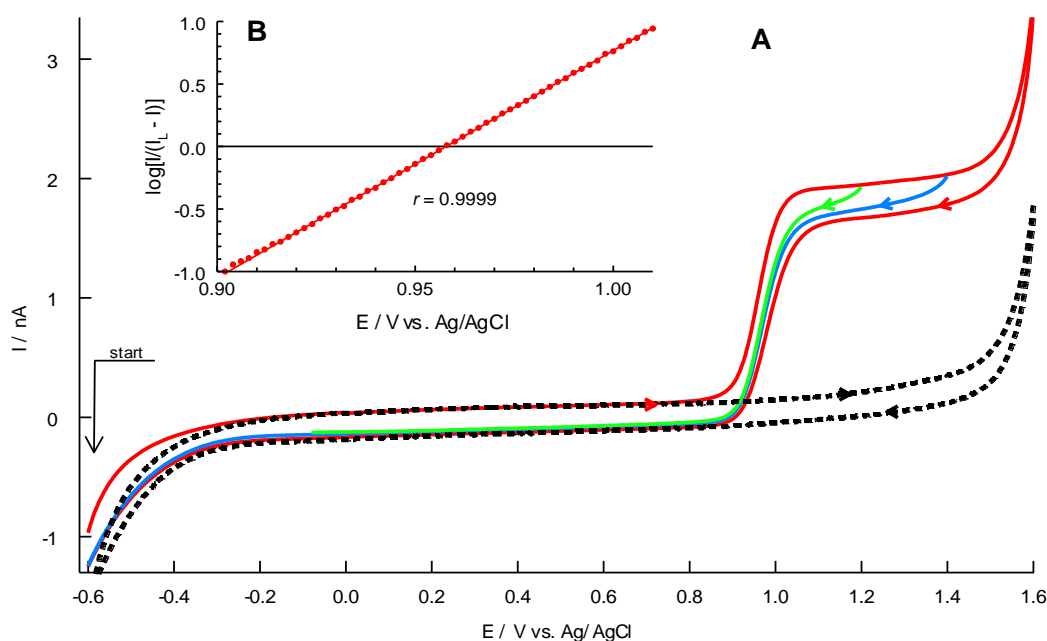

Figure 3. (**A**) LSVs (*v* = 5 mV s^{-1}) of the anodic LA oxidation (0.1 mM) on a CF. Direction of polarization was reversed from anodic to cathodic at potentials, E_λ: 1.2, 1.4, 1.6 V. Dashed line is residual current. (**B**) Semi-logarithmic analysis of the curve presented in panel (**A**)

In order to ensure steady-state conditions, voltammograms were recorded at a scan rate of 5 mV s^{-1}. A very well-shaped single anodic wave with a clearly marked steady-state limiting current was obtained in such conditions (Fig. 3(A)). LA oxidation proceeds at the potentials which exceed approximately 0.9 V vs. Ag/AgCl. An unlimited increase in current above 1.5 V is related to the

oxidation of the supporting electrolyte components, most probably acetate ions (dashed line). After reversing the direction of polarization from anodic to cathodic, a small hysteresis was observed. Its size decreases with the decreasing of the potential switching (E_λ). This may mean that the oxidation process is accompanied by only small changes on the surface of the electrode. Their source may be the adsorption of the electrode reaction products. The Tomes criterion was used to check the reversibility of electron exchange reactions. For a reversible process, the difference between the potentials corresponding to ¾ and ¼ of the steady-state current ($E_{3/4}$-$E_{1/4}$) depends on the number of the exchanged electrons, n, and at 25°C, should be 0.0564/n V [29,30]. The experimentally obtained value of 0.056 V (Table 1) indicates that the anodic LA oxidation process is close to reversible and proceeds with the exchange of one electron.

This conclusion is confirmed by the data obtained from a semi-logarithmic analysis of the LSV curve (Fig. 3(B)). According to the equation [29,30]:

$$E = E_{1/2} + 0.0591/n \log [I/(I_L - I)] \tag{3}$$

where, E – electrode potential [V], $E_{1/2}$ – half-wave potential [V], I – current [A] at a given potential E, I_L – steady-state limiting current [A], a linear dependence of $\log[I/(I_L-I)] = f(E)$ ($r = 0.9999$) was obtained with a slope, S of 0.055 V (Table 1). This value is close to the expected one in the case of a reversible one-electron process (0.0591/n V (temp. 25°C) [29,31]). The semi-logarithmic analysis can also be used for the precise estimation of the half-wave potential, $E_{1/2}$, which in the case of LA oxidation is 0.958 V (Table 1).

Table 1. Voltammetric parameters of anodic oxidation of LA obtained by LSV and DPV on CF microelectrode ($\Phi = 33$ μm) and by CV on GCE ($\Phi = 3$ mm).

	Technique			
	LSV ($v = 5$ mV s^{-1})	DPV (d$E = 20$ mV)	CV	
			($v = 5$ mV s^{-1})	($v = 100$ mV s^{-1})
Parameter	[1)]$E_{1/2}$ V vs. Ag/AgCl	[4)]E_p V vs. Ag/AgCl	[4)]E_p V vs. Ag/AgCl	
	0.958	0.965	0.932	0.976
	[2)]$E_{3/4}$-$E_{1/4}$ / V	[5)]$W_{1/2}$ / V	[6)]E_p-$E_{p/2}$ / V	
	0.056	0.090	0.051	0.066
	[3)]S / V	-	[7)]S / μA s V^{-1}	
	0.055		0.46	

[1)]half-wave potential, [2)]Tomes criterion, [3)]slope of the semi-logarithmic analysis expressed as dE/d(log[$I/(I_L-I)$]), [4)]peak potential, [5)]peak width at a half-height, [6)]criterion of reversibility in CV technique, [7)]slope of the relationship log(I_p, μA) = f(log v, V s^{-1})

In order to check whether the electrode process is controlled by diffusion or adsorption, LSV curves were recorded on microelectrodes with different radius (Fig. 4(A)). According to the equation [30,32]:

$$I_L = 4nFrDc \tag{4}$$

where: n – number of electrons exchanged in the electrode reaction, F – Faraday constant [C mol^{-1}], D – diffusion coefficient of the analyte [m^2 s^{-1}], c – bulk concentration of the analyte [mol m^{-3}], steady-state limiting current, I_L [A] should increase with the increasing radius of the electrode, r [m]. Due to the lack of a sufficient number of electrodes made of carbon fiber, platinum electrodes were also used. It was found that the microelectrode material did not affect the course of the recorded curves, but their reproducibility on platinum was unsatisfactory. The obtained linear relationship $I_L = f(r)$ (Fig. 4(B)) indicates that the process is controlled by diffusion.
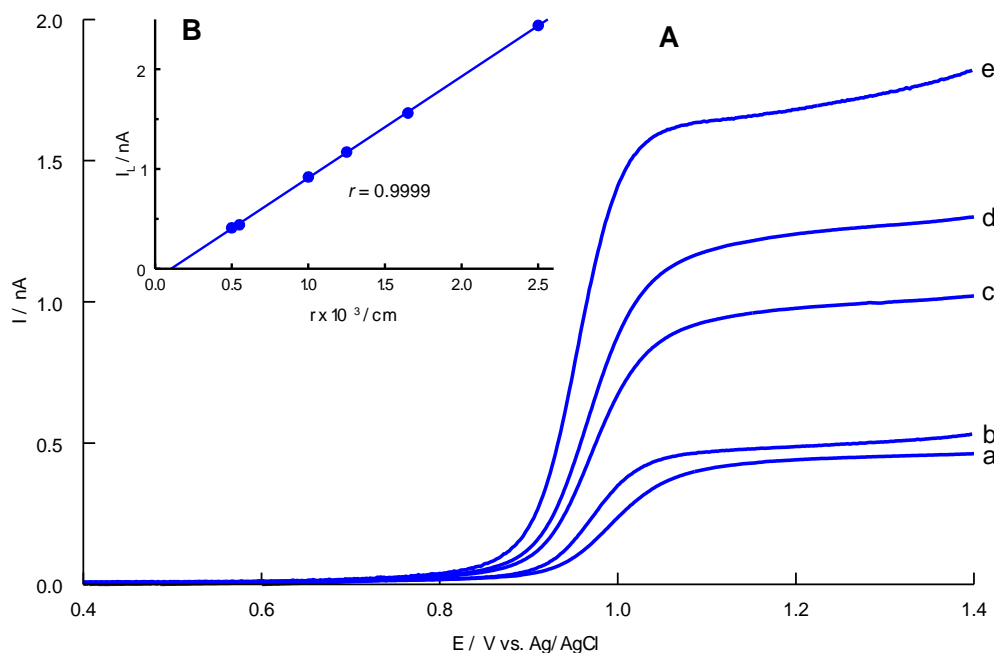

Figure 4. (**A**) LSVs of LA (0.1 mM) recorded on Pt with diameters: (a) 10; (b) 20; (c) 25; (e) 50 μm, and (d) CF ($\Phi = 33$ μm). (**B**) Dependence $I_L = f(r)$.

In the next stage of the research, the effect of concentration on the course of LSV curves recorded on the CF microelectrode was checked. For this purpose, a series of solutions containing LA in the concentration range of 0.02 - 1.00 mM was prepared. Fig. 5(A) shows representative LSVs obtained in the solutions containing different concentrations of LA. As can be seen, these curves are well-shaped, and their half-wave potential ($E_{1/2}$) is concentration independent. Based on the data from Fig. 5(A), a linear course of relationship $I_L = f(c)$ (Fig. 5(B)) was obtained. This confirms the possibility of using the LA oxidation process in quantitative analysis.

Since the electrode process is diffusion-controlled, equation (4) allowed to calculate the diffusion coefficient, D, which is $(1.20 \pm 0.01) \times 10^{-9}$ m^2 s^{-1}. This value is similar to the one obtained on GCE in phosphate buffer at pH 6.9 ($D = 1.1 \times 10^{-9}$ m^2 s^{-1}) [19].

In the LA oxidation research on microelectrodes, the DPV technique was also used. The relative position of the LA anodic oxidation curves in the studied environment recorded by the LSV and DPV techniques is shown in Fig. 6(A).
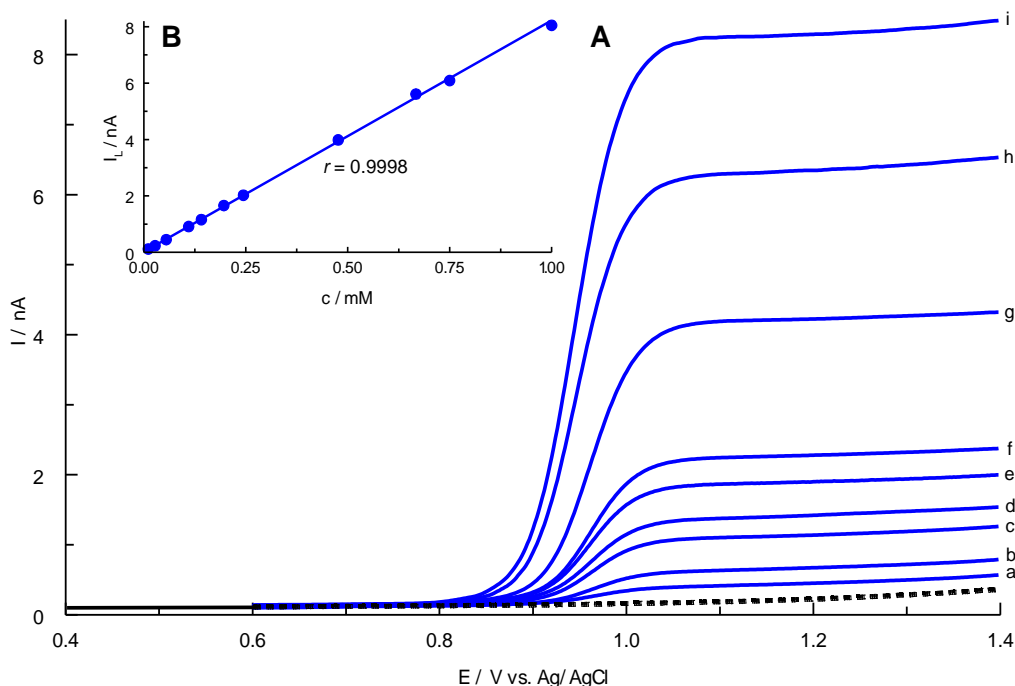
Figure 5. (**A**) LSVs (v = 5 mV s^{-1}) on a CF at different concentration of LA ranging from (a) 0.02 to (i) 1.0 mM. Dashed line is residual current. (**B**) Calibration plot for LA.

The use of DPV allows us to limit the adsorption phenomena on the surface of the working electrode, and to obtain high sensitivity and resolution of signals. The DPV curves have a characteristic well-shaped peak at the potential, E_p close to the half-wave potential, $E_{1/2}$ characteristic of the LSV technique. The difference between these potentials is small, and in the case of a reversible process it depends on the value of pulse amplitude (dE) [33]:

$$E_p = E_{1/2} + dE/2 \tag{5}$$

The peak potential (E_p) of the curves recorded by DPV is about 0.965 V (Table 1), and differs from $E_{1/2}$ by 7 mV. This value is close to 10 mV, which results from the applied amplitude (dE = 20 mV) and shows the compatibility of both potentials.

A characteristic parameter of the DPV curves is the peak width at half height, $W_{1/2}$ which for a reversible process can be described by the equation [31]:

$$W_{1/2} = 3.52 \, RT/nF \tag{6}$$

The theoretical value of $W_{1/2}$ should be 0.0904/n V at 25°C. The experimentally obtained value of 0.090 V (Table 1) is close to the theoretical one and confirm the reversibility of an electrode reaction which proceeds with the exchange of one electron.

The influence of LA concentration on the DPV curves is shown in Fig. 6(B). In each case very good reproducibility of the successively recorded voltammograms was observed. It is much better than in the case of the LSV technique. It also confirms the limitation of the influence of adsorption phenomena on signals recorded using pulse techniques. As in the case of the LSV curves, a linear dependence of I_p = f(c) was obtained (Fig. 6(B')). This confirms the applicability of the DPV technique for the determination of LA.
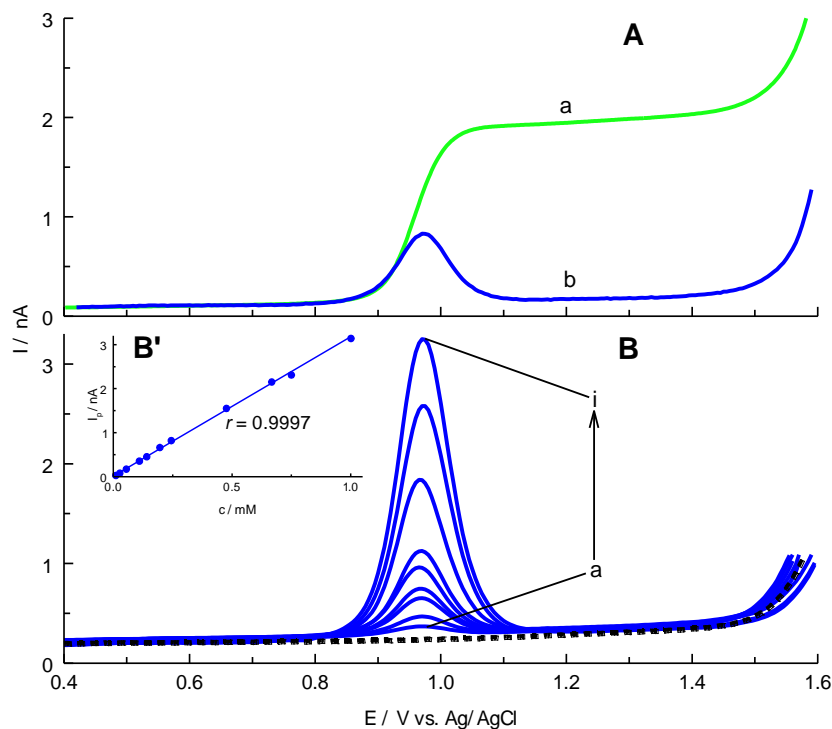
Figure 6. (**A**) Comparison of (a) LSVs ($v = 5$ mV s^{-1}) and (b) DPVs (d$E = 20$mV) of the anodic oxidation of LA (0.1 mM) recorded on a CF. (**B**) DPVs recorded at concentration of LA in the range from: (a) 0.02 to (i) 1.0 mM. (**B'**) Calibration curve.

3.2.2 Cyclic Voltammetry

Cyclic voltammetry (CV) was used to obtain further information on the mechanism of the anodic oxidation of α-lipoic acid, and in particular, on the products formed during this reaction. A glassy carbon macroelectrode (GCE, $\Phi = 3$ mm) was applied for this purpose. A higher concentration of the supporting electrolyte (0.5 M) was used to avoid a negative influence of ohmic potential drops (*IR*) between the electrodes associated with higher currents compared to those recorded on microelectrodes. An increase in the electrode diameter caused a change from spherical to linear diffusion, which appears as a peak on the registered curves. Well-shaped CV curves were recorded, and the LA oxidation peak was observed at the potential of above 0.9 V vs. Ag/AgCl (Fig. 7). This is close to $E_{1/2}$, and E_p, observed on the LSV and DPV curves respectively (Table 1). An unlimited increase in current above 1.5 V relates to the oxidation of the supporting electrolyte components.

When the direction of polarization (E_λ) was reversed, no reduction peak was observed. It did not even appear when E_λ was near the anodic oxidation peak. This means that the primary product of the anodic oxidation of LA is unstable. This product participate in the successive homogeneous reaction near the electrode surface, and it transform into non-electroactive products or those that were not reducible within the available range of potentials characteristic of the environment studied. No reduction peaks were observed on the curves recorded at higher potential scan rates (Fig. 8(A)).
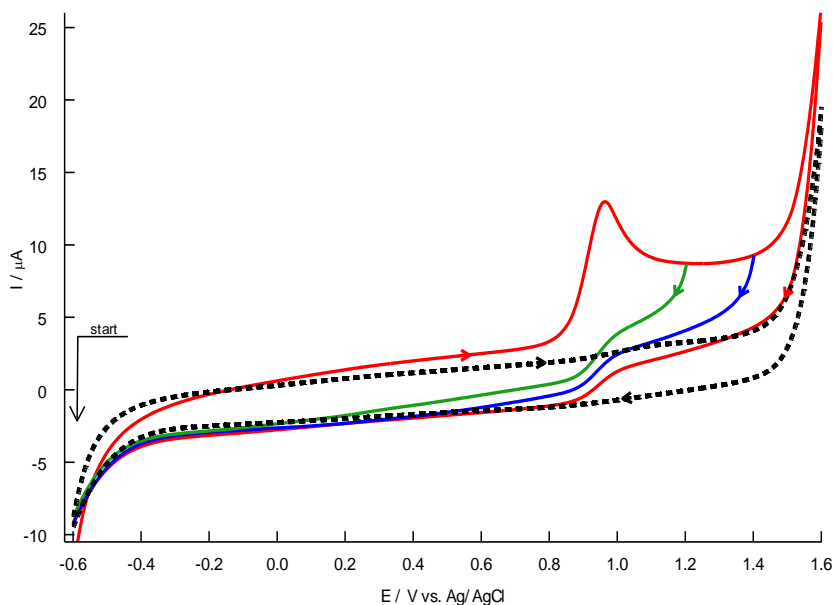
Figure 7. CVs ($v = 50$ mV s^{-1}) for LA oxidation (0.5 mM) on a GCE. Direction of electrode polarization was reversed from anodic to cathodic at potentials $E_\lambda = 1.2$; 1.4; 1.6 V. Dashed line is residual current.
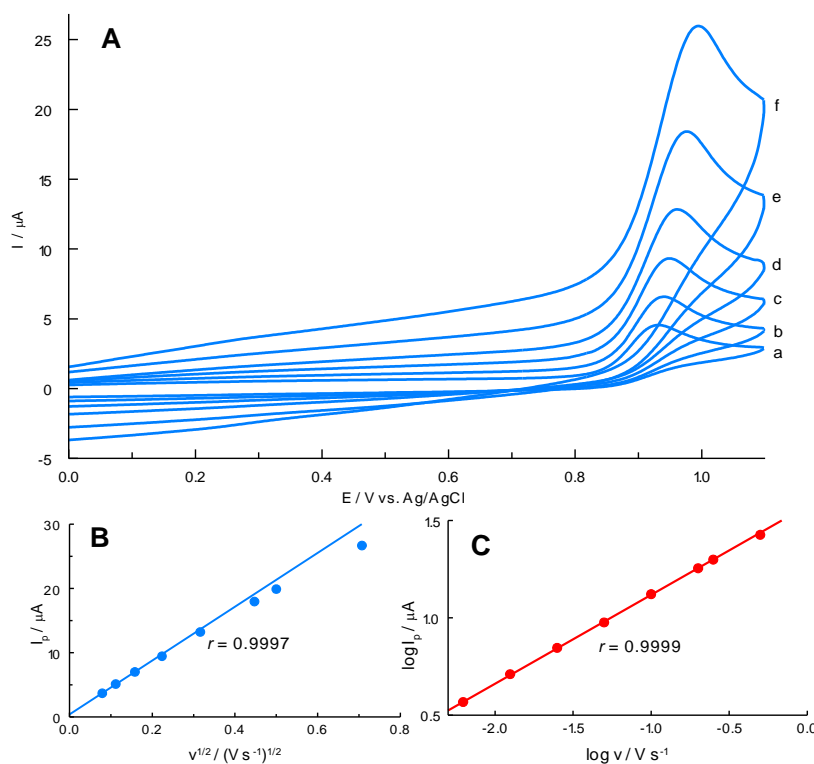

Figure 8. (**A**) CVs on GCE at different scan rates: a) 5; b) 12.5; c) 25; d) 50; e) 100; f) 200 mV s^{-1} for 0.5 mM LA. (**B**) Relationship between anodic peak currents, I_p, and square root of scan rate, $v^{1/2}$ (**C**) The dependence $\log (I_p) = f(\log (v))$.

In order to check the reversibility of the electrode process, one of the criteria based on the difference between the peak potential and potential corresponding to ½ of the peak current ($E_p - E_{p/2}$) was applied. The theoretical value characteristic for a reversible process is at 25°C 0.0564/n V [32]. The

experimental value is close to the theoretical one but increases with the applied scan rate, v, from 0.051 V to 0.066 V (for $v = 5$ and 100 mV s^{-1}, respectively, Table 1). This confirms the previous conclusions about the quasi-reversible character of the electrode process, which takes place with the exchange of one electron. An additional argument is a slight shift in the peak potential towards the anodic direction with increasing of scan rate (Fig. 8(A), Table 1). As can be seen from the CV curves presented in Fig. 8(A), the peak current I_p increases with an increase in v. The dependence of $I_p = f(v^{1/2})$ is described by the Randles-Sevcik equation [29]:

$$I_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D^{1/2}\, c\, v^{1/2} \tag{7}$$

where, A – electrode area [cm^2]. A linear relationship was obtained at small values of v, (Fig. 8(B)). This confirms that the electrode reaction is reversible and diffusion controlled. The deviation from the linearity observed at higher scan rates indicates an increase in the irreversibility of the LA oxidation process. The linear dependence $\log I_p = f(\log v)$ presented in Fig. 8(C) confirms the diffusion character of this process. The slope of this relationship (0.46) is close to the theoretical one (0.5) characteristic for pure diffusion electrode process.

3.2.3. Mechanism of the Anodic Oxidation of LA

The results of the study show that the anodic oxidation of α-lipoic acid on carbon electrodes in the proposed environment proceeds with the exchange of one electron. Additionally, this process is quasi-reversible, diffusion controlled, and its products are unstable. Therefore, it can be stated that the overall process proceeds according to the E_qC_i mechanism. Since the dissociation constants of LA (pK_a = 4.70 [3,14]), and acetic acid (pK_a = 4.75 [28]) are comparable, the undissociated molecule undergoes an electrode reaction. This oxidation process occurs on one of the sulfur atoms of the dithiol ring. The quasi-reversible exchange of one electron (E_q) leads to the formation of an unstable cation radical. The differences in charge density on the sulfur atoms obtained by PM3 method in the HyperChem program (Fig. 9) show that the loss of the electron proceeds from the sulfur atom closer to the carbon chain. The resulting cation radical, as an electrode reaction product, undergoes successive irreversible, homogeneous reactions (C_i). Consequently, the final products are formed which are non-electroactive or non-reducing in the available potentials range, and thus are difficult to identify.
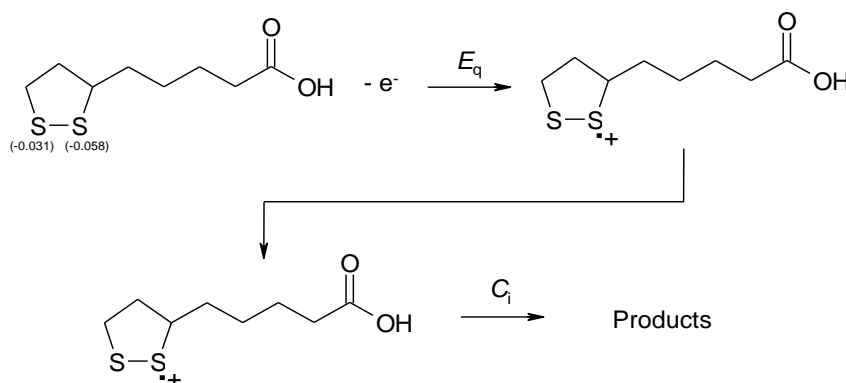

Figure 9. LA oxidation mechanism proposed. The electron densities on sulfur atoms are given in brackets.

4. CONCLUSION

A mixture of acetic acid and acetonitrile (20%, *v/v*) containing sodium acetate as a supporting electrolyte is a convenient environment for studying the electrochemical properties of α-lipoic acid. The LA oxidation process takes place at the potential of about 0.96 V vs. Ag/AgCl, it is quasi-reversible, controlled by diffusion and proceeds with the exchange of one electron according to the E_qC_i mechanism. The observed linear dependence of the oxidation current on the concentration of LA in the studied environment and very good reproducibility of the curves, those recorded by DPV in particular, indicate that this electrode process can be used to develop a new voltammetric method for the determination of this analyte in pharmaceutical preparations.

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