Voltammetric Determination of α-Lipoic Acid using Carbon Fiber Microelectrode in Acetic Acid – Acetonitrile Solutions

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The work aimed to develop a new voltammetric method for the identification and determination of α-lipoic acid in pharmaceutical preparations. In our previous article, we proved that a mixture of acetic acid and acetonitrile (20%, v/v), containing of 0.1 M CH_3COONa as a supporting electrolyte, as well as differential pulse voltammetry (DPV) on a carbon fiber microelectrode are convenient for voltammetric study of the electrochemical properties of LA. The oxidation process of α-lipoic acid, characterized as quasi-reversible and diffusion controlled, proceeds with the exchange of one electron giving unstable cation radical as primary product (E_qC_i mechanism). This electrode reaction was the basis for developing a new voltammetric method of LA determination with the use of both the DPV technique, and the multiple standard addition method. The proposed procedure makes it possible to determine LA without the need to separate it from the matrix and it has a wide linear range (0.25-123.2 mg L^{-1}) and low detection limit (0.08 mg L^{-1}). Additionally, it is accurate, precise, and can be applied for LA determination in pharmaceutical products as an alternative to widely used chromatographic methods.

1. INTRODUCTION

α-Lipoic acid (LA, Fig. 1) is a natural compound found in the world of plants and animals [1]. Thanks to its biochemical and, in particular, antioxidant properties, it has found a comprehensive therapeutic application. LA is used in the treatment of diseases related to oxidative stress such as cardiovascular diseases [2], multiple sclerosis [3], Alzheimer's disease [4], type 2 diabetes, diabetic neuropathy [5,6], obesity [7] and liver diseases caused by poisoning with alcohol, mushroom or heavy metals [8,9]. It can also mitigate the effects of anti-cancer therapies [1,6], AIDS or HIV [6,10]. In pharmaceuticals and dietary supplements, LA is mainly used as a substance supporting neurological processes and proper functioning of the nervous system or regulating the carbohydrate metabolism. This

compound exists in the human organism in the form of free acid or proteins bounded LA by weak hydrogen bonds or strong covalent bonds (lipoyllysine) [11].
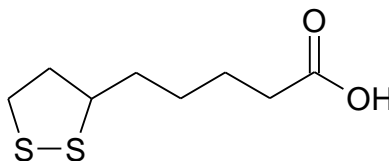

Figure 1. Molecular structure of α-lipoic (LA).

Broad dissemination and significant biochemical properties of LA make it important to monitor its content in real samples and during metabolic changes in living organisms. It can be determined both as a free acid and bounded to lipoyllysine [12-16]. Various analytical techniques are used for quantitative LA analysis in biological matrices (plant and animal tissues) and in pharmaceutical preparations - in medicines or in food supplements. These are mainly: high performance liquid chromatography (HPLC) with various detection methods - chemiluminescence [17], spectrophotometric UV-Vis [18,19], fluorescence [20], electrochemical [21], and gas chromatography (GC) with flame photometric detection [22], or mass spectrometry [23], as well as spectrophotometry [24-27], capillary electrophoresis (CE) [28,29], coulometry [30,31] or voltammetry [9,32-36].

It is evident that among all the analytical techniques, chromatography occupies a dominant position. Its main advantages include selectivity, high sensitivity, and low detection limits. However, high equipment costs and a complex and long-lasting sample preparation process often requiring derivatization of the analyte, mean that alternative methods are sought, such as electrochemistry and, in particular, voltammetry. These techniques are characterized by the analytical parameters comparable to these of chromatographic ones. Unlike chromatography, however, they do not demand expensive equipment. Another asset is the simplicity of sample preparation for analysis, which shortens the time of this important stage, especially in the case of non-biological matrices. In the course of voltammetric analysis of electroactive substances, derivatization is not necessary. Sample preparation is often limited to its dissolution in a suitable solvent [9,32], and optionally to filtration separating insoluble matrix components [32]. Their use may limit the presence of other electroactive components of a sample, the signals of which may interfere with the analyte signal [9,32-34]. However, selectivity can increase by changing measurement conditions, for instance, those of a measuring technique, material of working electrode or composition of a solvent and/or a supporting electrolyte. Various electrode materials, including platinum [9], glassy carbon (GCE) [32], boron-doped diamond electrode (BDDE) [33], fluorine-doped tin oxide (FTO) [34], pyrolytic graphite electrode modified with cobalt phthalocyanine (PG/CoPc) [35], manganese(IV) oxide-modified screen-printed graphene electrode (MnO$_2$/SPGE) [36] have been applied for the voltammetric determination of LA so far. Most often the content of α-lipoic acid has been tested in different aqueous buffer solutions: phosphate [32,35], acetate [9], Britton-Robinson [33] or in their mixtures with alcohols [32,36]. Organic solvents have been rarely used for this purpose.

Corduneanu *et al.* [32] applied a simple procedure of sample preparation in the form of a dietary supplement, which included its dissolution in a mixture of water-methanol (1:1, v/v) and filtration of the

solution. 0.1 M phosphate buffer (pH 6.9) was used as a supporting electrolyte. The basis for the determination of LA was its oxidation peak at the potential of 0.8 V vs. Ag/AgCl observed on DPV curves recorded on glassy carbon working electrode. Unfortunately, the signals derived from other electroactive substances such as ascorbic and uric acid, or glucose, interfered with the signal of the analyte, which affects the quality of the assay. The analytical parameters, such as linearity range (0.5-15 $\mu g\ mL^{-1}$), and limits of determination (*LOD*) and quantification (*LOQ*) (0.37 and 1.3 $\mu g\ mL^{-1}$ respectively), and the recovery (*R*) at the level of 98.2% proved practical usefulness of this method.

Changing the conditions of the analysis makes it possible to eliminate the influence of the accompanying substances on the analyte signal. Marin *et al.* [9] described the procedure of free LA determination in human plasma using a platinum disk as a working electrode. Sample preparation was limited only to dissolution of a blood sample in a solution of 0.2 M acetate buffer (pH 4.5) in a volume ratio of 1:24. They found out that the compounds existing in the plasma, such as ascorbic or uric acid, did not affect the course of DPV oxidation curves for LA. The authors concluded that the method could be used in the concentration range of 2-165 $\mu g\ mL^{-1}$, and *LOQ* value was at the level of 10.84 $\mu g\ mL^{-1}$. The procedure proved to be simple, reliable, and useful for precise and sensitive analyzes in routine clinical trials on diseases related to oxidative stress.

When determining LA in urine samples, Stankovic *et al.* [33] did not observe a negative impact of the signals derived from uric and ascorbic acids on the LA oxidation peak. The signals were recorded on a boron-doped diamond electrode (BDDE) using the DPV technique. A well-shaped oxidation peak in a Britton-Robinson buffer at pH 3 was observed at the potential of about 0.9 V vs. Ag/AgCl. Dopamine also oxidizes in a similar range of potentials, which makes it difficult to determine LA. By changing pH to 6, the resolution of the signals increased. Under these conditions, the *LOD* value was 0.018 $\mu g\ mL^{-1}$, and it was clearly lower than those obtained on GC [32] and Pt [9] electrodes. The linear dependence of the peak current on the concentration of the analyte appeared. However, it was in a much narrower range than in the solutions of the acetate buffer on the platinum electrode [9] and was within the range of 0.062-21.63 $\mu g\ mL^{-1}$ [33].

For LA determination in plasma or urine samples, Miranda *et al.* [34] used the process of LA oxidation in a solution of sulfuric acid(VI) at pH 2, using square wave voltammetry (SWV) on a fluorine-doped tin oxide electrode (FTO). The authors stated that this environment was not beneficial for that purpose because the uric and ascorbic acids present in the samples were oxidized near the peak potential of the analyte (0.95 V vs. Ag/AgCl). It is possible, however, to analyze LA content in pharmaceutical preparations in the presence of riboflavin, folic acid or vitamin B12.

The LA oxidation peak observed on the DPV curves, recorded on a pyrolytic graphite electrode modified with cobalt phthalocyanine (PG/CoPc) was the basis for the determination of this analyte in dietary supplements [35]. Phosphate buffer (pH 7.0) was a supporting electrolyte. A very narrow linearity range (0.1-3.9 $\mu g\ mL^{-1}$) was observed in comparison to those obtained with the use of other electrodes [9,32-34]. However, the limits of detection and quantification were significantly lower (0.7×10^{-3} and 2.5×10^{-3} $\mu g\ mL^{-1}$, respectively). The multiple standard addition method was used for the quantitative analysis of LA. The recovery of the analyte was within the limits of 99.0-103.0%.

Charoenkitamorn *et al.* [36] chose another type of electrode material for the determination of LA in dietary supplements. The measurements were carried out on the manganese(IV) oxide-modified

screen-printed graphene electrode (MnO$_2$/SPGE), using square wave anodic stripping voltammetry (SWASV). LA was determined in the presence of Q10 coenzyme. A mixture of ethanol and acetate buffer at pH 4.0 (20:80, v/v) was used as a reaction medium to increase the solubility of both analytes. According to the authors, the method is beneficial for the simultaneous determination of LA and Q10 with good sensitivity and repeatability. The determined linearity range of the method was within 0.3-25.0 µg mL^{-1}, and the *LOD* was at the level of 0.088 µg mL^{-1}.

Based on the literature review, it appears that organic solvents have not been used as a medium for the determination of LA until now. Due to the limited solubility of LA in water (0.127 g L^{-1} [37]), it is desirable to search for a different solvent. Our previous studies on the electrochemical properties of LA [38] have shown that the optimal environment for this purpose can be a mixture of acetic acid and acetonitrile (20%, v/v) containing 0.1 M CH$_3$COONa as a supporting electrolyte. The composition of the solution allows the dissolution of both the analyte and the matrix present in formulations containing LA, and also the hydrophilic supporting electrolyte, which is indispensable in voltammetric measurements. It is also important that acetic acid has a wide range of anodic stability [39] and is safe for human and the environment. In contrast, the presence of acetonitrile ($\varepsilon = 35.9$ [40]) increases the electric conductivity of the solutions and facilitates charge transfer between the electrodes and reduces the contribution of adsorption phenomena. It has been proved that the process of LA oxidation on a carbon fiber microelectrode (CF) in the proposed solution takes place at the potential of about 0.96 V vs. Ag/AgCl. It is diffusion-controlled and proceeds with a quasi-reversible exchange of one electron giving unstable cation radical as a primary product (E_qC_i mechanism) [38].

The purpose of this work is to apply the oxidation process of LA described above in the acetic acid environment to develop a new voltammetric method for its determination in pharmaceutical preparations.

2. EXPERIMENTAL

2.1 Chemicals and reagents

The following reagents were used in the studies: (±)-α-lipoic acid (LA, ≥99%, Sigma-Aldrich), CRS lipoic acid (99.9%, European Pharmacopoeia (EP) Reference Standard, Sigma-Aldrich), sodium acetate (AcNa, anhydrous>99.0%, Fluka), glacial acetic acid (AcH, p.a. ACS, Merck), acetonitrile (AN, p.a. ACS>99.5%, Merck). Various types of medicinal products available on the Polish pharmaceutical market were selected for the experiments. They had a different formulation: a coated tablet (Thiogamma, Wörwag Pharma GmbH & Co. KG, Germany), soft capsules (Alfalipon prodiab, BIOTON S.A., Poland) and hard capsules (Liponexin, SOLINEA sp. z o.o., Poland).

2.2 Instrumentation

The determination of α-lipoic acid in pharmaceutical preparations was carried out using the M161 digital electrochemical analyzer and the M162 pre-amplifier for measuring small currents. Both

instruments were cooperating with the mEALab 2.1 software (mtm-anko, Cracow). We used a three-electrode cell, in which the working electrode was a carbon-fiber microelectrode (CF, $\Phi = 33$ μm). This optimal electrode material for LA oxidation was selected at the stage of studying its electrochemical properties [38]. The potential was measured against an external silver chloride reference electrode with 1 M NaCl solution (Ag/AgCl). This electrode was placed in a salt bridge with a frit of Vicor Glass filled with a supporting electrolyte. The platinum wire was an auxiliary electrode. The electrolytic cell with the electrodes was placed in the Faraday cage, thus limiting the influence of external factors on the measured signals. Differential pulse voltammetry (DPV), as a measurement technique chosen, provided good resolution of the signals, as well as excellent reproducibility of the successively recorded curves. Limiting the participation of adsorption phenomena of the oxidation products was significant for the results. The optimized parameters for the DPV technique were as follows: the pulse amplitude ($dE = 20$ mV), the pulse width of 80 ms, and the scan rate of 20 mV s^{-1}. Such parameters guaranteed to obtain a signal from LA of the highest intensity, which, in turn, allowed to achieve high sensitivity and low *LOQ*. The peak width at half height $W_{1/2}$, affecting the resolution of the signals, was also taken into account. A narrow signal of the analyte allows its identification even in the presence of accompanying electroactive components. During the optimization process, we observed an increase in the peak current, along with an increase in the pulse amplitude and $W_{1/2}$. The applied amplitude is a compromise between the appropriate sensitivity of signals and their resolution. The pulse width did not affect the peak current significantly, but its value of 80 ms ensured perfect repeatability of the successively recorded curves.

3. RESULTS AND DISCUSSION

3.1 Interferences study

The determination of LA in real samples began with checking whether the remaining ingredients of the preparations also dissolved in the proposed environment and whether their presence affected the recorded oxidation of the analyte peak current. The preparations also contained other compounds that enhance the effect of the analyte, mainly vitamins B (B1, B2, B6), vitamin E, selenium, zinc, chromium (Table 1). In addition to biologically active substances, there were also fillers, stabilizers and anti-caking agents (not included in Table 1).

Table 1. The main components found in the tested preparations.

PREPARATION		
ALFALIPON prodiab	Thiogamma	Liponexin
Purpose		
Dietary supplement	Medicament	Food for special medical purposes
Components	**Formulation**	

	Soft capsule	Coated tablet	Hard capsule
α-lipoic acid (LA)	350 mg	600 mg	300 mg
γ-linolenic acid (GLA)	6.3 mg	-	-
Niacin	12 mg niacin eqv.	-	-
Vitamin B2	1.4 mg	-	-
Vitamin B6	1.4 mg	-	-
Vitamin B1	1.1 mg	-	-
Vitamin E	-	-	10 mg
Zinc	7.5 mg	-	-
Chromium	40 μg	-	-
Selenium	-	-	50 μg

The preliminary stage of the study was to check the possibility of identifying the signal derived from LA. Also, we intended to determine the influence of the matrix components on the oxidation signal of the analyte. To these ends, the DPV curves were recorded on the carbon fiber microelectrode (CF, Φ 33 μm), in solutions containing LA and vitamins B2, B1, B6 and E (each with a concentration of approx. 6 mg L^{-1}). As can be seen from Fig. 2, vitamin B1 (curve *b*) does not oxidize in the studied environment. In contrast, vitamin B2 voltammogram (curve *c*) gives a complex signal in the potential range of -0.35 to 0.08 V vs. Ag/AgCl, which does not affect the course of the curve related to the oxidation of the analyte (curve *a*). Well-shaped oxidation peaks can be observed for vitamins E (curve *d*) and B6 (curve *e*) at potentials of 0.56 V and 1.07 V vs. Ag/AgCl, respectively. The location of the oxidation signal derived from vitamin E significantly differs from the one characteristic for the oxidation of α-lipoic acid (E_p = 0.965 V vs. Ag/AgCl). Partial overlap of the oxidation peaks is observed in the case of vitamin B6 and LA. The presence of this vitamin may impede the determination of the analyte. To address the problem, we examined the effect of adding vitamin B6 on the peak current of LA.
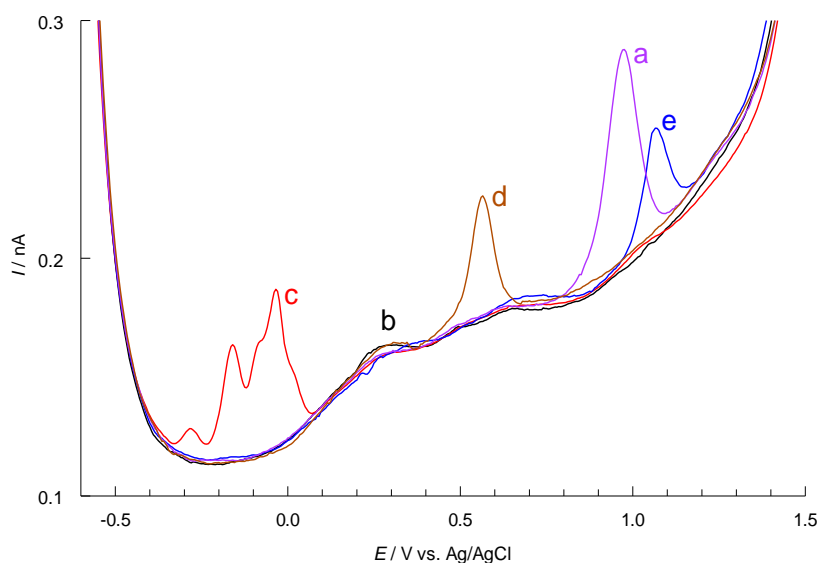

Figure 2. DPVs of LA (*a*), vitamins: B1 (*b*), B2 (*c*), E (*d*), B6 (*e*) (each is concentration of approx. of 6 mg L^{-1}) recorded on CF (Φ = 33 μm) in a mixture of AcH and AN (20%, v/v) containing 0.1 M AcNa.

For this purpose, DPV curves were recorded in solutions containing LA (Fig. 3, curve *a*) and in mixtures of LA and vitamin B6 with comparable concentrations (Fig. 3, curve *b*) and twice the amount of this interferent (Fig. 3, curve *c*). As can be easily noticed, only the double excess of vitamin B6 has a significant influence on the course of the curves and peak current, which increases by 13% in comparison with the observed one in the absence of this interferent. Considering that the amount of vitamin B6 in the pharmaceuticals studied is at the level of 0.4%, this substance cannot affect the accuracy of LA determination.
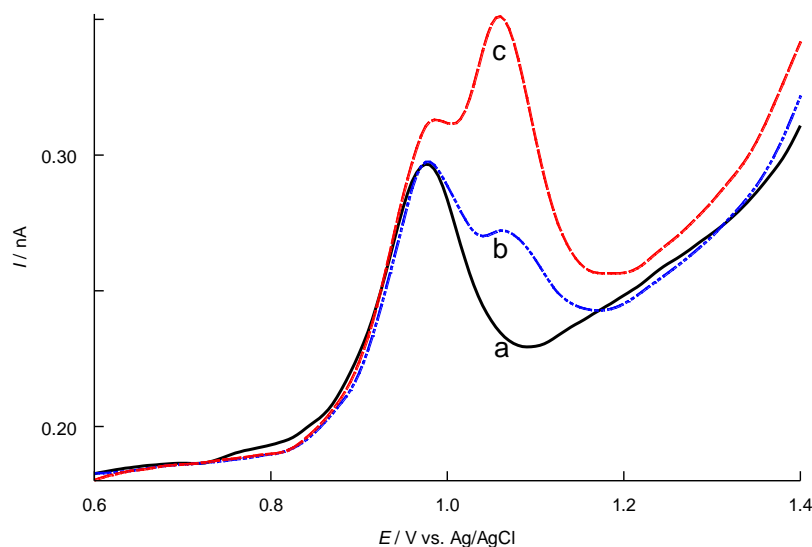

Figure 3. DPVs recorded on CF (Φ = 33 μm) in solutions containing (*a*) LA (6.01 mg L^{-1}), (*b*) as (*a*) with vitamin B6 (6.20 mg L^{-1}), (*c*) as (*a*) with vitamin B6 (12.30 mg L^{-1}).

The remaining ingredients of the preparations also do not affect the oxidation signal of the analyte, either. The confirmation of this is the course of the curves recorded in the solutions containing pharmaceutical preparations with the same amounts of the analyte (about 6 mg L^{-1}). We can observe a single and well-shaped signal with a maximum at the potential of 0.965 V vs. Ag/AgCl (Fig. 4). Its location is consistent with the signal recorded in LA standard solution (Fig. 4, curve *a*). The lack of other signals on the presented curves shows that the remaining components of the preparations are at a concentration level that does not modify the analyte signal.

Worth noting is very good repeatability of the successively recorded DPV curves in solutions containing the investigated pharmaceuticals. This means that the adsorption phenomenon does not accompany the LA oxidation process on the working electrode surface. Therefore, there was no need to polish it during the voltammetric analysis, which prevented errors related to the loss of the analyte.
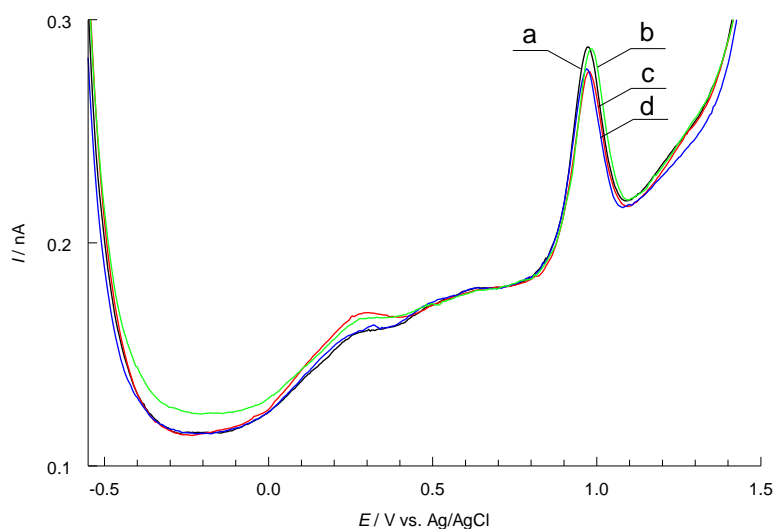
Figure 4. DPVs recorded on CF (Φ = 33 μm) in AcH containing AN (20%, *v/v*), 0.1 M AcNa and (*a*) LA (6.01 mg L^{-1}), pharmaceutical preparations (each solution contained LA in concentration about 6 mg L^{-1}): (*b*) Liponexin, (*c*) Alfalipon, (*d*) Thiogamma.

3.2 Validation of the method

The determination of LA began with the method validation and defining basic analytical parameters: linearity range, limits of detection (*LOD*) and quantification (*LOQ*). The first step was to check how the change in concentration affects the size of the measured signal. A series of standard solutions with different analyte concentrations ranging from about 0.1 to 200 mg L^{-1} was prepared. An increase in the peak current of the DPV curves recorded in those solutions was observed. (Fig. 5A). The change in concentration did not affect the signal position on the potential axis. The recorded curves enabled to construct a calibration plot and to determine the linearity range of LA (Fig. 5B) and other analytical parameters (Table 2).
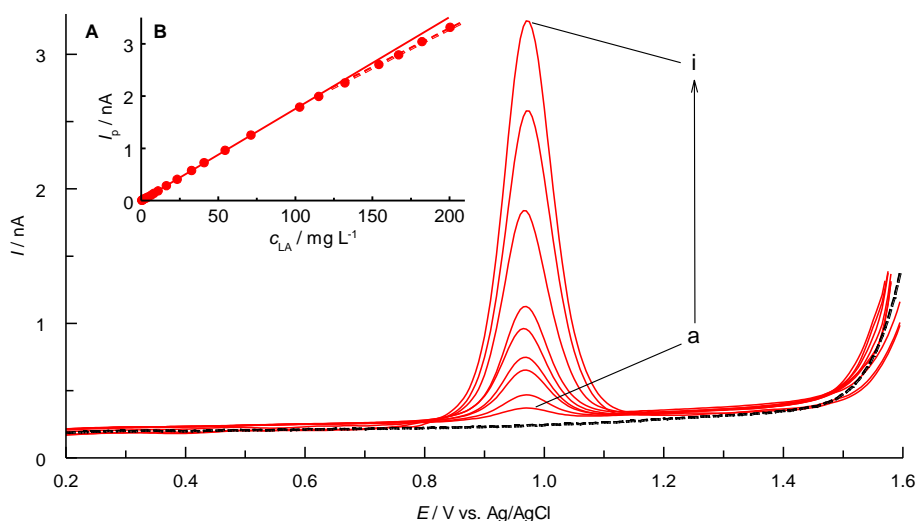

Figure 5. (**A**) DPVs recorded on CF (Φ = 33 μm) in solution containing different concentration of LA: (*a*) 0.1 - (*i*) 200.4 mg L^{-1}. (**B**) Calibration plot for LA.

The limits of detection (*LOD*) and quantification (*LOQ*) were determined based on the standard deviation of the blank, σ_B and the slope of the calibration plot, a_1 ($LOD = 3.3\sigma_B /a_1$, $LOQ = 5\times LOD$) [41,42]. The obtained results indicate that the voltammetric LA determination method is characterized by a wide range of linearity and by a low *LOD* and *LOQ* values (Table 2).

Table 2. Peak potential (E_p) and the parameters of the calibration plot, I_p (nA) $= a_0 + a_1c$ (mg L^{-1}) obtained using DPV to the oxidation of LA on CF ($\Phi = 33$ μm) in AcH containing AN (20%, *v/v*), 0.1 M AcNa.

Parameter	LA
E_p / V vs Ag/AgCl	0.965
Linearity range / mg L^{-1}	0.25 – 123.2
Regression coefficient, r	0.9999
$a_0 \pm$ [1]S_{a0} / nA	-0.001 $\pm$ 0.002
$a_1 \pm$ [1]S_{a1} / nA mg^{-1} L	0.018 $\pm$ 8$\times10^{-5}$
LOD / mg L^{-1}	0.08
LOQ / mg L^{-1}	0.39

[1] Standard deviation of the intercept (a_0) or the slope (a_1) of the calibration plot

Comparing the validation parameters of the developed method with the literature data (Table 3), one can be concluded that the proposed analytical procedure allows achieving a wider range of linearity [32-36] and lower limits of detection and quantification [9,33]. The exception is the procedure for LA determination in the blood plasma [9] with a slightly wider range of linearity, but higher values of *LOD* and *LOQ* (Table 3). A lower detection limit was achieved only on BDDE and on PG/CoPc electrode, but the linearity ranges were much narrower [33].

Table 3. Comparison of the analytical parameters of voltammetric LA determination based on selected literature data and own research.

Method (electrode material)	Sample type	Linearity range μg mL^{-1}	*LOD* μg mL^{-1}	*LOQ* μg mL^{-1}	*R* %	Ref.
DPV (GCE)	Dietary supplements	0.5-15	0.37	1.3	98.2	[32]
DPV (Pt)	Plasma	2-165	2.71	10.84	[1]nd	[9]
DPV (BDDE)	Biological samples	0.062-21.63	0.018	nd	95-97	[33]
SWV (FTO)	Dietary supplements	1.03-41.2	0.76	2.53	99.3-104.1	[34]

DPV (PG/CoPc)	Dietary supplements	0.1-3.9	0.7×10^{-3}	2.5×10^{-3}	99.0-103.0	[35]
SWASV (MnO$_2$/SPGE)	Dietary supplements	0.3-25.0	0.088	nd	95.2-103.2	[36]
DPV (CF)	**Medical products**	**0.2-123.2**	**0.08**	**0.39**	**97.5-100.9**	**This work**

[1] nd – no data

The presented results of the preliminary tests and the validation of the method allow stating that the proposed analytical procedure is suitable for the voltammetric determination of α-lipoic acid in pharmaceutical preparations.

3.3. Voltammetric determination of α-lipoic acid in pharmaceutical products

The initial stage of the quantitative analysis of α-lipoic acid in pharmaceuticals included verifying the reliability of the method. For this purpose, we compared the amount of the analyte introduced into the control solution with the one obtained during the analysis, using the multiple standard addition method. The initial volume of the test solution was 2.0 mL, and the LA standard solution was added in portions of 50 μL. The determinations were carried out at four LA concentration levels. We chose an optimal concentration of the standard to obtain appropriate peak current increments, and the total LA concentration was within the linearity of the method. DPV curves were recorded in the initial solution and after each addition of the standard one. Five measurements were made for each determination.

Table 4. Results of the voltammetric determination of LA in Pharmacopoeia Reference Material and in control solutions.

	Added mg L^{-1}	[1] Found mg L^{-1}	[2] R %	[3] RSD %
		Pharmacopoeia Reference Material		
	5.78	5.77 ± 0.03	99.8	0.35
No.		Control solutions		
1	2.88	2.89 ± 0.09	100.4	2.58
2	5.76	5.82 ± 0.05	101.0	0.68
3	11.52	11.68 ± 0.30	101.5	2.10
4	23.04	23.00 ± 0.54	99.8	1.88

[1] $x = x_{av} \pm t_{0.95} S x_{av}$ for $n = 5$ and $t_{0.95} = 2.776$ (tabulated), $S x_{av}$ – standard deviation of the mean,
[2] $R = (x_{av} / added) \times 100\%$, [3] Relative standard deviation

The amount of analyte in the solution was determined using the mEAlab 2.1 program, based on the peak current dependence on the concentration. We compared the obtained concentrations with those introduced. The recovery (R) of 99.8-101.5% was obtained. Small differences between the results of the subsequent measurements and a small value of relative standard deviation, (RSD 0.68-2.10%) prove the method to be precise. The lowest RSD value was for the LA concentration of 5.76 mg L^{-1} (Table 4).

To confirm the reliability of the results, a solution containing Pharmacopoeia Reference Material with a concentration of 5.78 mg L^{-1} was prepared. This was similar to the concentration of the control solution with the lowest RSD. The result was accurate ($R = 99.8\%$) and precise ($RSD = 0.35\%$). The differences between the recoveries obtained in the control assay and using the reference material (Table 4) may result from the purity of the reagents used. The purity of the compound used to prepare the control solutions was 99%, while of the α-lipoic acid as reference material was 99.9%.

The results discussed above prove that the proposed analytical procedure is reliable and appropriate for the determination of LA in real preparations. The tested pharmaceuticals were in different formulation. Because of this, the sample preparation procedures differed one from another. In the case of Thiogamma, which was in the form of a coated tablet, it consisted of crushing and grinding several portions in an agate mortar. After opening the Alfalipon soft capsules, their content was spilled. Next, a portion of each pharmaceutical underwent dissolving in 50 mL acetonitrile. As for Liponexin, a hard gel capsule with liquid content, was cut open, and then its content was dissolved in 100 mL acetonitrile. Next, we took the appropriate amount of acetonitrilic solution so that after dilution with the mixture of acetic acid and acetonitrile (20%, v/v) containing supporting electrolyte, the concentration of LA allowed recording DPV curves with the initial peak current of about 0.3 nA. The curves were recorded in initial solutions, and after the addition of four portions of the standard (Fig. 6A.). The LA content in the sample was determined in the same way as in the case of control solutions. Fig. 6B shows the calibration plots of the multiple standard addition methods for all five LA determinations in the Alfalipon preparation. Other pharmaceuticals followed the same procedure.
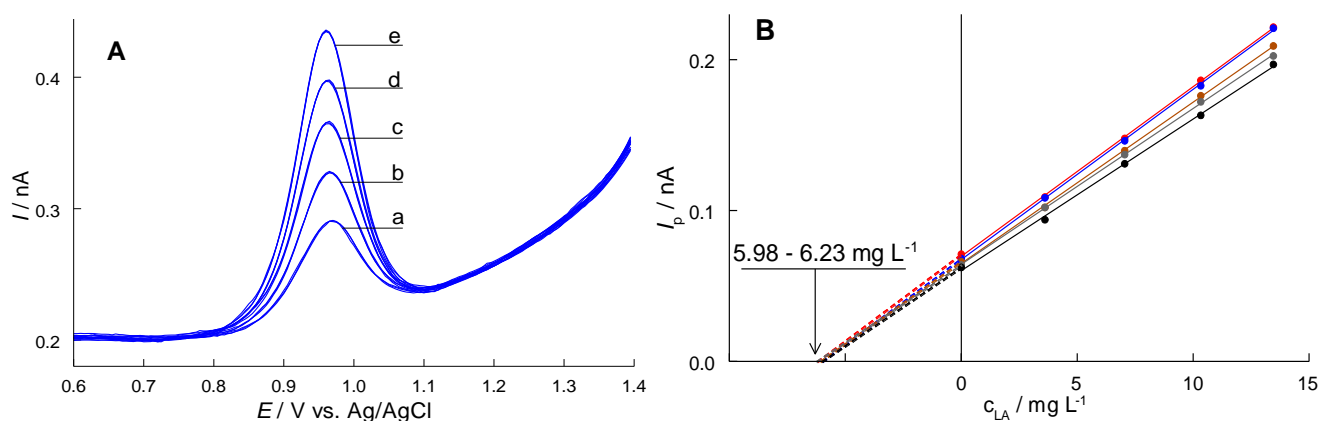

Figure 6. (**A**) DPVs recorded in a solution containing (*a*) Alfalipon ($V_0 = 2.0$ mL) and after addition of the LA as a standard solution (148.0 mg L^{-1}) with the volume of: (*b*) 50 μL, (*c*) 100 μL, (*d*) 150 μL, (*e*) 200 μL. (**B**) Calibration plots for 5 determinations in the standard addition method.

Table 5 presents the results of the voltammetric determination of α-lipoic acid in the pharmaceuticals tested. We obtained a very good agreement between the experimentally determined LA contents and those declared by the manufacturers. That was confirmed by recovery values in the range of 97.5-100.9%. This range is narrower than those obtained by other voltammetric methods using various types of electrode materials (Table 3). The proposed method is also more accurate than other presented in the literature (Table 3). This may be due to a simpler sample preparation procedure which allows to shorten the determination time, minimize potential errors and lower the costs of analysis and reduce the amount of reagents used. The advantageous of acetic acid - acetonitrile used as a medium is the possibility of dissolving both the hydrophobic analyte and the matrix present in the preparation investigated. In addition, this environment prevents the blocking of the electrode surface, which ensures very good reproducibility of DPV curves and thus of the final results. The smallest recovery was obtained for Thiogamma, probably due to the analyte losses during grinding and weighing the sample. The results obtained for Liponexin were the most accurate and precise. We based the sample preparation procedure for this pharmaceutical only on dissolving the contents of the entire capsule, including its coating. This way, we eliminated the weighing stage of the sample, which is likely to cause errors.

Table 5. Results of the voltammetric determination of LA in pharmaceutical preparations.

Preparation	LA content / mg in a serving		[2]R %	[3]RSD %
	declared	[1]determination		
ALFALIPON prodiab	350	353.2 ± 8.5	100.9	1.94
Thiogamma	600	585.1 ± 17.5	97.5	2.40
Liponexin	300	300.2 ± 0.3	100.1	0.89

[1]legend such as in Table 4.

The relative standard deviation, in the range of 0.89-2.40% indicates that the developed method is precise. These values were comparable to obtained by other authors (Table 3).

5. CONCLUSION

The presented results show that the proposed mixed solvent: acetic acid and acetonitrile (20%, v/v) containing 0.1 M sodium acetate as a supporting electrolyte, is a convenient environment for the voltammetric determination of LA in pharmaceutical preparations. The basis for the procedure is the LA oxidation process occurring on a carbon fiber microelectrode (CF) with a diameter of 33 μm. Differential pulse voltammetry (DPV) and the multiple standard addition method were used for the purpose of LA determination. The proposed analytical procedure is characterized by a wide range of linearity and low values of limits of detection (*LOD*) and quantification (*LOQ*). The preparation of samples for analysis is simple and quick, which allows saving reagents and reducing the cost of the analysis. The developed method is accurate as confirmed by values recovery (97.5-100.9%) and precise (*RSD* 0.89-2.40%), and

can be an alternative to other voltammetric and chromatographic methods in the quantitative analysis of LA carried out in pharmaceutical laboratories.

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