Anal. Bioanal. Electrochem., Vol. 12, No. 6, 2020, 857-869

Analytical& Bioanalytical Electrochemistry

2020 by CEE
www.abechem.com

Full Paper

Voltammetric Determination of α-Lipoic Acid using Poly(Vanillin) Modified Platinum Electrode

Serap Titretir Duran,[1] Chedia Ben Ali Hassine,[2] Muammer Burç,[1] and Öznur Güngör[1,*]

[1]*Chemistry Department, Arts and Sciences Faculty, İnönü University, 44280, Malatya-Turkey*

[2]*Electrical and Electronics Engineering Department,Engineering Faculty, Özyegin University, İstanbul-Turkey*

*Corresponding Author, Tel.: +90-422-3773873; Fax.: +90-422-3410037

E-Mail: oznur.gungor@inonu.edu.tr

Received: 3 May 2020 / Received in revised form: 24 June 2020 / Accepted: 24 June 2020 / Published online: 30 June 2020

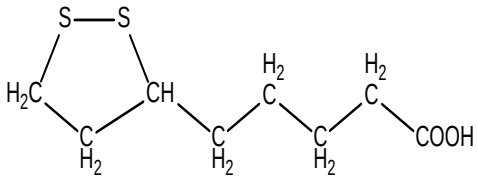
Abstract- In the present study electrochemical detection of α-lipoic acid (α-LA) was carried out with a *poly*(Vanillin) modified platinum electrode *p*(VA)/PtE in Brinton Robinson buffer (BR) solution. The platinum electrode surface's was modified with cyclic voltammetry method. The prepared Pt electrode was characterized by cyclic voltammetry in ferrocyanide and scanning electron microscopy (SEM) images. The number of cycles and scan rate effects were studied using square wave voltammetry (SWV) in BR buffer containing 1 mM α-LA. The electrochemical oxidation of α-lipoic acid on the modified Pt electrode is influenced by the pH and it is an irreversible reaction in which one electron and one proton are transferred. Electrolyte type and pH effect were studied. The anodic peak current shows a linear increase with α-LA concentrations ranging from 0.03 mM to 2.00 mM at pH 5, the detection limit being of 0.025 mM. The α-lipoic acid quantification in synthetic urine samples was made by SWV with the modified Pt electrode.

Keywords- Square wave voltammetry; α-lipoic acid; Vanillin; Platinum electrode

1. INTRODUCTION

α-lipoic acid (α-LA) is an antioxidant also named 1,2 dithiolane-3-pentanoic acid. Scheme 1 shows the chemical structures of α-LA. α-LA can be synthesized by animal, plant

and human cells from fatty acids and cysteine. α-LA is very important for human health since it help prevent oxidative stress [1-3] thanks to its antioxidant properties. Food and cosmetics industries use α-LA as preservative. Substantial interest has been given to the use of α-LA because of it's therapeutic abilities in so many diseases like diabetes, hepatitis, radiation damage, cardiovascular diseases, HIV infections, mitochondrial cytopathies, neurovascular abnormalities and heavy metal poisoning [4-7]. Furthermore, α-LA is used in the treatment of both chemical and mushroom poisoning [1].



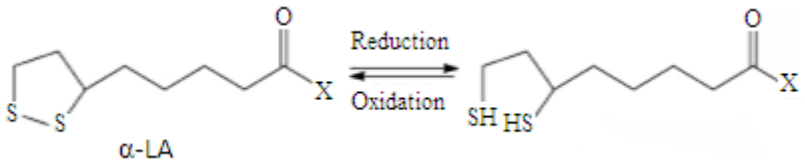
Scheme 1. Chemical structures of α-LA

The determination of α-LA concentration in biological fluids has been attracting different research groups' attention because the quantification of its level in biological fluids can give information about people's health if we consider the correlation between α-LA level and chronic diseases occurrence. In addition, α-LA level is important in pharmaceutical and cosmetic products for quality control testing [8].

Until now several methods have been described for α-LA detection such as liquid chromatography with high performance liquid [9], mass spectrometry, gas chromatography, liquid chromatography [10], spectrophotometry [11], fluorescence [12], chemiluminescence [13], coulometry [14] and capillary electrophoresis [15]. However, these methods have a quite high cost and are complex, troublesome and time demanding. Also, most importantly, they require an excessive amount of samples. Besides, The presence of interfering species in most of these methods can often make them inappropriate [16]. For all these reasons, there is still a need to develop new, selective and sensitive methods that are not affected by interferent substances and that are less costly, simpler, effortless, less time-consuming and also require less sample size.

Electrochemically modified electrodes are convenient for environmental, clinical and biotechnical analyzes. They are conceived to provide the required selective regions towards the analytes [17-20]. Electrochemically modified electrodes have made the use of electrochemical methods interesting due to their selectivity, precision and ability to reach very low detection limits. In addition, electrochemical methods attract attention in literature because they provide faster response, reliability, simplicity, simple instrumentation, improved precision, low-cost methodology and a small amount of samples. Therefore, electrochemical methods have great advantages over other commonly used analytical methods [21-23].

A redox reaction takes place in the determination of α-LA. It is shown in Scheme 2.



Scheme 2. The α-LA oxidation and reduction structure [24]

Many efforts have been made in order to develop a sensitive sensor for α-LA determination, such as functionalized multi walled carbon nanotubes (MWCNT) polyindole/Ti_2O_3 nanocomposite modified glassy carbon (GC) electrode [25]. Furthermore, a-lipoic acid was voltammetrically detected in human serum. This was applied on bare platinum (Pt) electrode in acetate buffer solution [8] and on carbon electrodes in acetic acid and acetonitrile solutions using differential pulse voltammetry method [26]. According to this work, vanillin (VA) was electro-polymerized onto platinum electrode and used for electrochemical detection of α-LA. As far as we know there is no report about the electrochemical determination of α-LA using the *poly*(VA) modified Pt electrode. Vanillin also known as 4-hydroxy-3-methoxybenzaldehyde is a chemical compound gaining increasing interest in different research areas as a potential monomer or precursor [27]. Food industry are using Synthetic vanillin as a flavoring agent. It is also used in medicines production [28]. In this work, *poly*(VA) platinum electrode was employed for a sensing determination of α-LA using square wave voltammetry. The application of the modified electrode in synthetic urine samples has been investigated.

2. EXPERIMENTAL

2.1. Apparatus

The reagents were used of analytical purity and purchased. α-lipoic acid and Triton X-100 were purchased from Sigma Aldrich. Vanillin, Brinton Robinson Buffer (NaOH, CH_3COOH, H_3PO_4, H_3BO_3, KCl), Ferricyanide and KNO_3 were purchased from Merck. H_2SO_4 were bought from Carlo Erba.

In all electrochemical experiments, a conventional three electrodes cell was used. The counter electrode used was a wire of platinum and the working electrodes used were platinum electrodes with 1.6 mm diameter. An Ag/AgCl/(sat. KCl) electrode was employed as the reference electrode. The electrochemical experiments were run with a potentiostat (Vertex one) controlled by computer with software (Ivium soft) for data analysis. Square wave measurement was used for α-lipoic acid detection and performed after 60 s pre-concentration step with an accumulation potential (E_{acc}) equal to -0.6 V under stirring. After the accumulation step was carried out, square wave measurements were registered from 0.5 to

1.2 V with a frequency equal to 100 Hz and an amplitude of 70 mV in the presence of Brinton Robinson (BR) Buffer 0.1 M (pH=5) containing 0.2% of TritonX-100.The morphological characterization of the modified electrode was carried out by a scanning electron microscope LEO-EVO 40 using the platinum working electrode. All glassware and electrochemical cells were kept in 6 M HNO_3 overnight and washed with deionized water in order to remove impurities. After that, they were dried in an oven. All electrochemical measurements were run at room temperature conditions.

2.2.1. Monomer solution preparation

1 mM of Vanillin was dissolved in 0.5 M H_2SO_4. For each measurement, fresh a solution was prepared. Total volume cell is 5 mL.

2.2.2. α-lipoic acid solution preparation

α-lipoic acid solution was dissolved in BR Buffer 0.1M (pH=5) with 0.2% of TritonX-100. Electrochemical measurements were carried out by adding the calculated amount of the analytes to the electrochemical cell.

2.2.3. Synthetic urine preparation

Synthetic urine was prepared by dissolving in deionized water 6.25 g of urea, 0.40 g of KCl, 0.56 g of Na_2SO_4, 0.25g of NH_4Cl, 0.35 g of KH_2PO_4, 0.28 g of $CaCl_2.2H_2O$ and 0.73 g of NaCl [29].

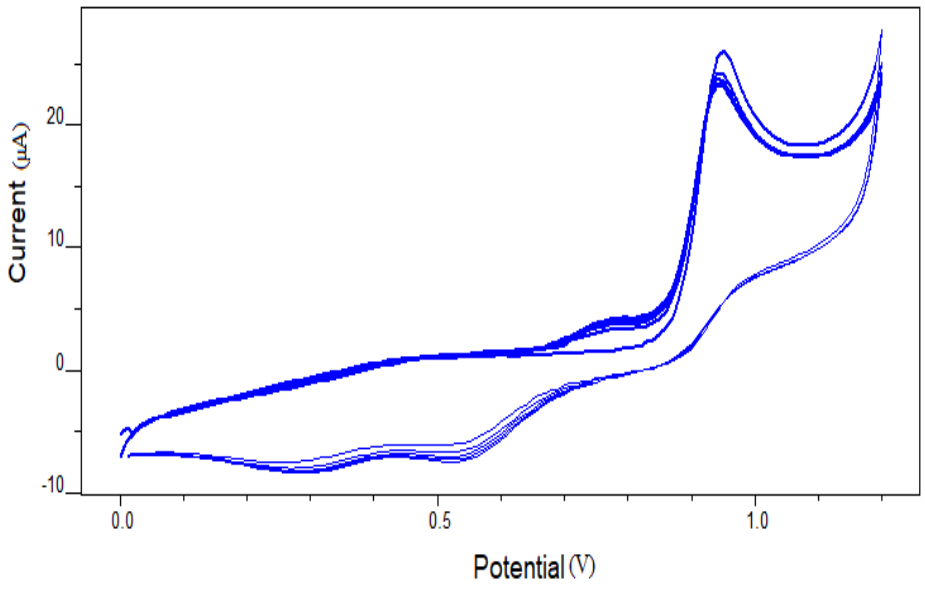


Fig. 1. Cyclic voltammogram electropolymerization of VA on platinum electrode in 0.5 M H_2SO_4, 5 cycle, scan rate 40 mV/s *vs.* Ag /AgCl/(sat.KCl)

2.2.4. Electrode modification procedure

The platinum electrode's surface was hand-polished with a 10 μm slurry of alumina water employing a polishing kit. To clean its surface, after being washed the polished electrode was sonicated in deionized water for 1 min. Then, the electrode modification was done using five reversible potential cycles between 0.0 V and +1.2 V in aqueous solution including 0.5 M H_2SO_4 at the scan rate of 40 $mV.s^{-1}$ (Fig. 1). The modified platinum electrode was characterized with scanning electron microscopy (SEM) and electrochemical methods.

3. RESULTS AND DISCUSSION

3.1. Optimization of the polymerization conditions

The modification of the platinum electrode was performed with Cyclic voltammogram vanillin in 0.5 M sulfuric acid at a scanning speed of 40 mV/s. Figure 1 displays the platinum electrode's surface cyclic voltammograms during the electropolymerization step. From Scheme 1, it is shown that the polymerization of VA is marked by the cyclic voltammograms variations recorded during the platinum electrode modification. An irreversible peak is recorded, in the first potential scan, at approximately +950 mV. This could be explained by the VA molecules adsorption on the electrode's surface. Thereafter, the current decreased in the cyclic voltammograms spectrum [30,31].

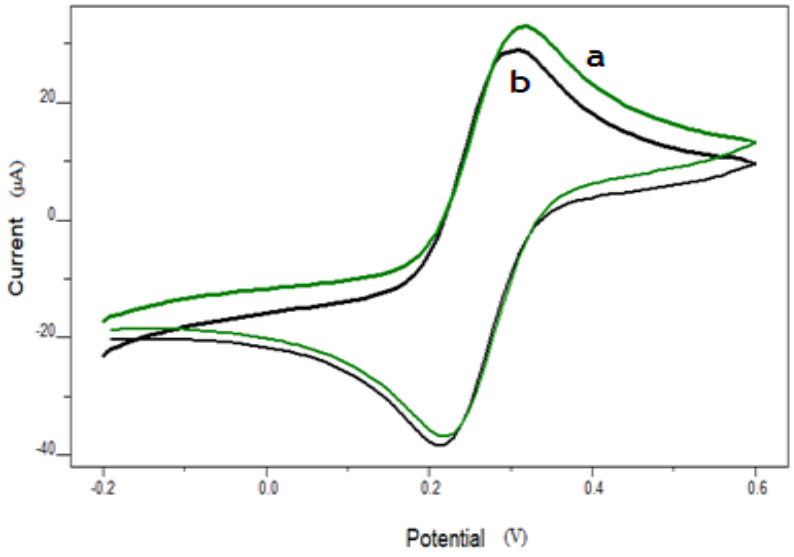


Fig. 2. The cyclic voltammograms of 6 mM ferrocyanide in 1 M KNO_3 of bare PtE **(a),** and Pt/*poly*(VA) **(b)**. At potential scan rate of 100 mV/s vs. Ag/AgCl/(sat. KCl)

Cyclic voltammetry was used to characterize the modified electrode. The cyclic voltammograms were registered in an aqueous solution of the redox compounds: $Fe(CN)_6^{4-}$, scan rate of 100 mV/s, in 6 mM of ferrocyanide containing 1 M KNO_3 in order to determine the creation of the VA polymer layer on the Pt electrode surface. Figure 2 exhibits the cyclic

voltammograms obtained for the modified and the bare Platinum electrode. The voltammograms of the redox medium display a reversible behavior with the two electrodes with a smaller separation peak for the bare electrode ΔE=70mV than for the modified electrode ΔE=100 mV. After the electrode surface's modification, there is an increase in current and a larger separation peak is registered for the *p*(VA)/Pt. This demonstrates the efficiency of the oxidation reaction of $Fe(CN)_6^{4-}$ with the *p*(VA) modified Pt electrode.

3.2. SEM study of *poly*(VA)

To characterize the electrodes, SEM of the bare platinum electrode (Figure 3A) and *p*(VA) (Figure 3B) on platinum electrode were given in Figure 3. The bare platinum electrode has a smooth surface with some roughness due to the electrode polishing. Figure 3B illustrates the dispersion of *p*(VA) on the electrode. Therefore, these differences can be explained by the creation of the polymeric thin layer on the surface of the electrode.

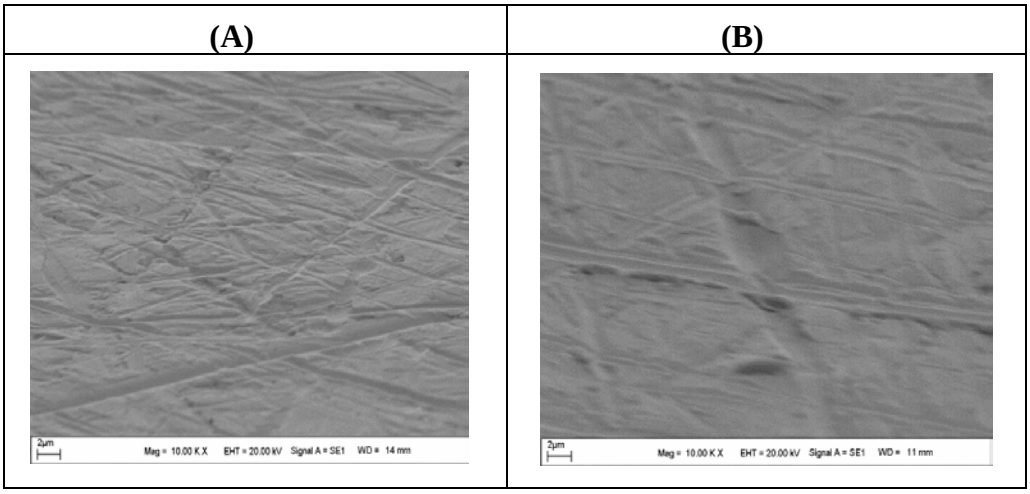


Fig. 3. SEM images of bare **(A)**, *poly*(VA) **(B)** onto Pt electrode

3.3. Determination of redox behaviours of α-lipoic acid

Cyclic voltammograms of alpha-lipoic acid in PBS (pH = 7.00) solution were taken to determine oxidation and reduction behaviours on both the platinum electrode and the *p*(VA) coated platinum electrode. As shown the Figure 4, the peak current of *p*(VA) coated platinum electrode higher than bare platinum electrodes'. The oxidation potentials of both electrodes are approximately 0.9 V.

From Figure 4, it can be predicted that modification of the Platinum electrode with *p*(VA) can significantly reduce the detection limit value of alpha lipoic acid.

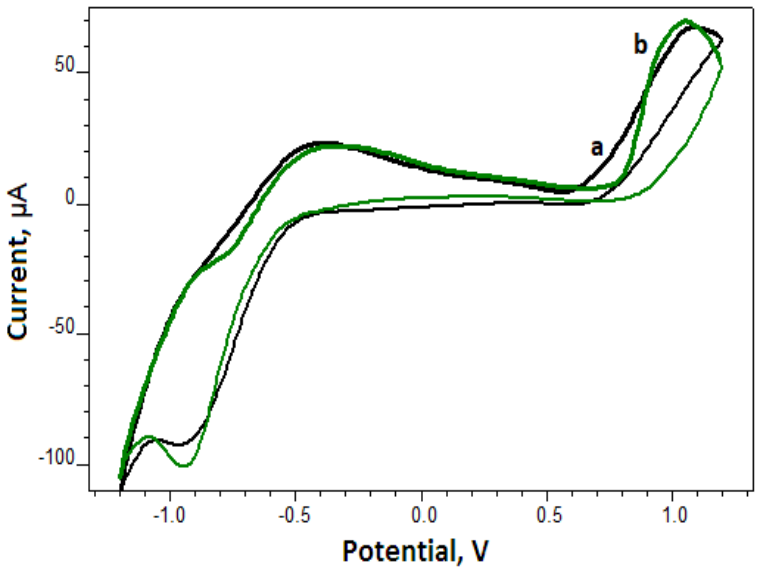


Fig. 4. The cyclic voltammograms of α-LA on bare **(a)**, *p*(VA) **(b)** onto Pt electrode in PBS (pH=7.00)

3.4. Effect of the cycle number, scan rate and pH

Optimization of the polymerization condition, the effect of the cycle number on the current response of 1 mM α-lipoic acid was tested by SWV method. Figure 5 displays the Results. The current response of the modified electrode increased as the cycle number value increased from 3 to 5 and then decreased for higher values. Consequently, 5 cycle were chosen for all experiments.

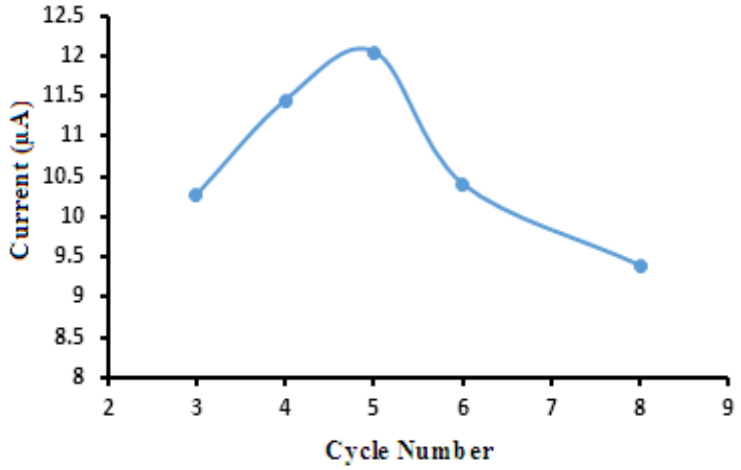


Fig. 5. Effect of cycle number on current oxidation of 1 mM α-lipoic acid

The scan rate's effect on the polymerization of VA was examined by SWV in the presence of 1 mM α-lipoic acid. The graph of current versus scan rate was drawn in the range of 20 to 70 mV/s. We observe an increase in the anodic peak current (I_{pa}) related to the increase of the scan rate until 40mV/s and then the anodic peak current decreased (Fig. 6)

(the increase of the scan rate caused the anodic peak current (I_{pa}) to increase as well until 40 mV/s and then the anodic peak current decreased). Thus, 40 mV was selected as the optimum scan rate value for the polymerization process.

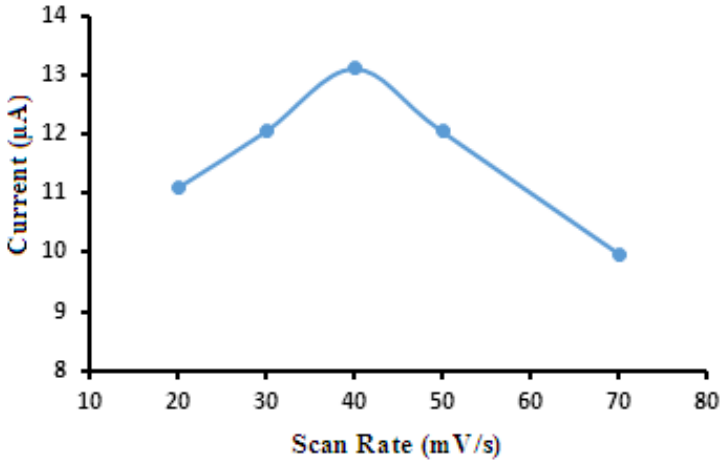


Fig. 6. Graph of current vs scan rate

Different parameters can affect the signal given by the modified electrode. For this reason, the effect of some of those parameters was studied such as electrolyte type, electrolyte pH, Amplitude, accumulation potential and accumulation time (t_{acc}) (Fig. 7 and 8).

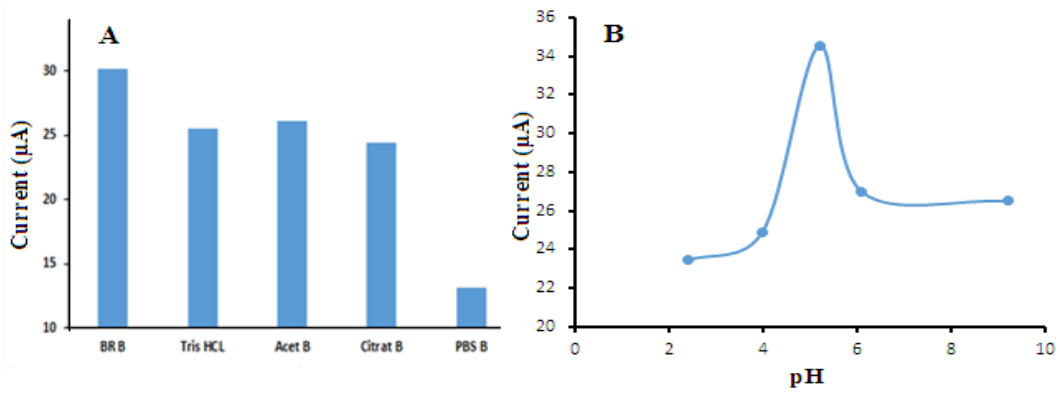


Fig. 7. Electrolyte type effect on DPV of 1 mM α-lipoic acid **(A)**, Relation between the anodic peak current and pH values in 0.1 M BR buffer **(B)**

Square wave voltammetry was used to study all those parameters in the presence of 1 mM α-lipoic acid. The electrolyte type's effect on the electroactivity of the modified electrode was studied and the results are given in Figure 7A. It has been observed from Figure 7A that the BR buffer gives a higher current response compared to the other buffer solution. Consequently, BR buffer as electrolyte type was chosen for the detection of α-lipoic acid. pH level of the buffer is also an important parameter and needed to be studied. Different pH levels of the BR buffer were studied in the range of 2.4- 9.2, as shown in Figure 7B. The

relation between pH and anodic peak potential is illustrated in Figure 7B. It can be seen that the current of the anodic peaks increases with the increase of pH values until pH reaches 5.00. This suggests that the α-LA electro-oxidation performance depends on pH and that the hydrogen ion's concentration disrupts the oxidation reaction's rate. Therefore, pH 5 was selected as the working pH condition for further electrochemical studies.

3.5. Effect of the amplitude, accumulation potential and time

The amplitude effect was examined between 10 and 100mV and the highest peak current was found for 50 mV as shown in Figure 8A. The accumulation potential was optimized in the range of 0.4 to 1.0 V. The highest SWV result was observed at E_{acc}=0.6 V (Fig. 8B). To determine the accumulation time's effect on the oxidation of α-LA, the SWV voltammograms were registered for the modified electrode in the presence of 1 mM α-LA in 0.1 M BR buffer at pH 5 as shown in Figure 8C. The α-LA oxidation peak currents were registered for different accumulation times between 40 and 120 s. The highest oxidation peak current was noted for 60 s. On that account, the appropriate accumulation time for the modified electrode optimization was estimated to a value of 60 s for an efficient α-LA molecule oxidation.

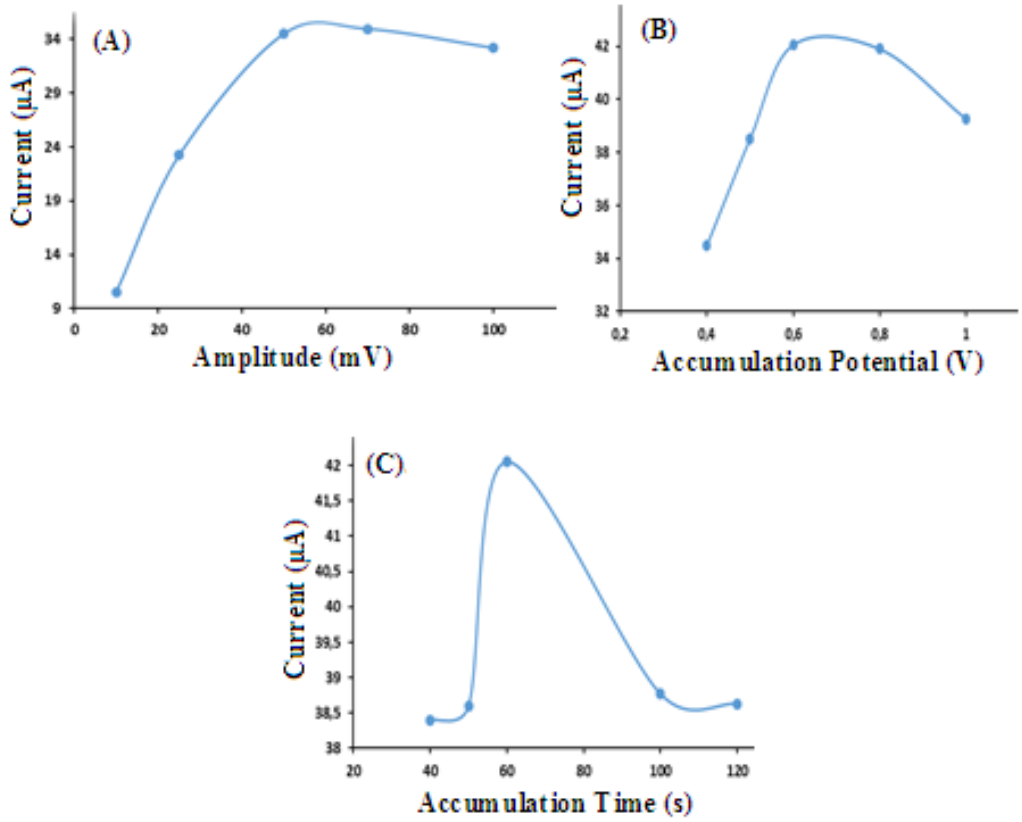


Fig. 8. SWV parameters effect**:** Amplitude **(A),** Accumulation Potential **(B**) and Accumulation Time **(C)**

3.6. Stability, Reproducibility sensitivity of the prepared electrode

Figure 9 demonstrates the stability of the *poly*(VA) modified Pt electrode. The magnitude of the SWV responses that are shown in Figure 9A and reproducibility in Figure 9B have been obtained with fifteen measurements. The stability and relative standard deviation of percent (%RSD) of the *poly*(VA) modified Pt electrode for 1 mM α-lipoic acid in 0.1 M BR buffer were calculated as 96.63% and 3.37% respectively.

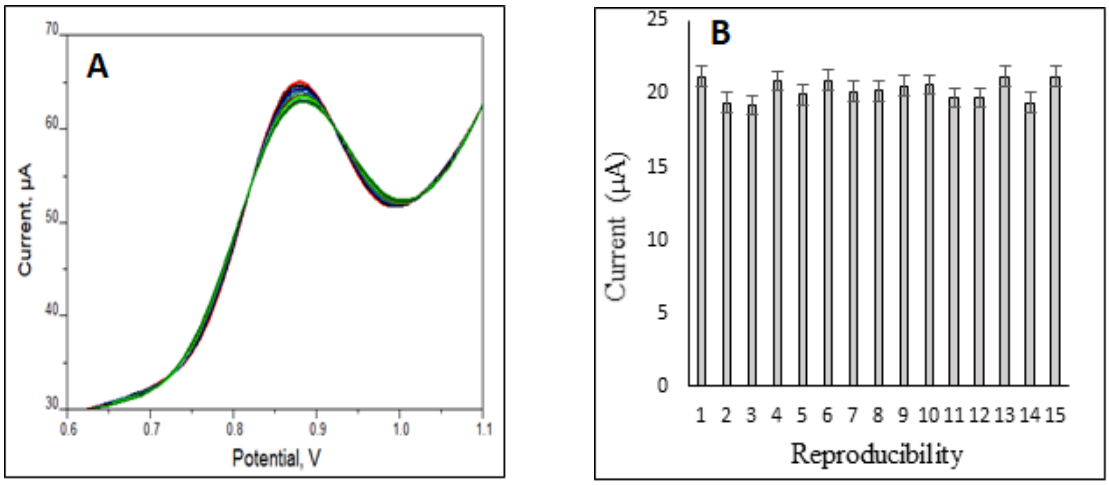


Fig. 9. Reproducibility of the SWV responses **(A)**, and error bars **(B)** at optimum conditions of α-lipoic acid on *poly*(VA) modified Pt electrode in 0.1 M BR at pH 5 (n=15)

3.7. Calibration curve and statistical analysis

Since square wave voltammetry is a high sensitive method if compared with cyclic voltammetry and differential pulse voltammetry, it was used, in this study, for α-lipoic acid detection. To check the sensitivity of the modified platinum electrode surface, SWV measurements were carried out in 0.1M BR buffer solution, pH=5.0. SWV voltammograms were investigated in different α-LA concentrations from 0.03 mM to 2.0 mM. Figure 7 displays square wave voltammograms for low α-lipoic acid concentrations (Fig. 10A), high α-lipoic acid concentrations (Fig. 10B) and the corresponding calibration plot (Fig. 10C). As shown in the Figure 10A and 10B, during the successive addition of α-lipoic acid, a clear peak response was noted, demonstrating the sensitivity of *poly*(VA) modified Pt electrode toward low and high α-lipoic acid concentration. The response current is linear between 0.03 mM and - 2.0 mM for α-lipoic acid. The calibration plot over the concentration has a slope with the correlation coefficient of 0.9999, quantitation limit (QL) of 0.075 mM and the detection limit (DL) of 0.025 mM.

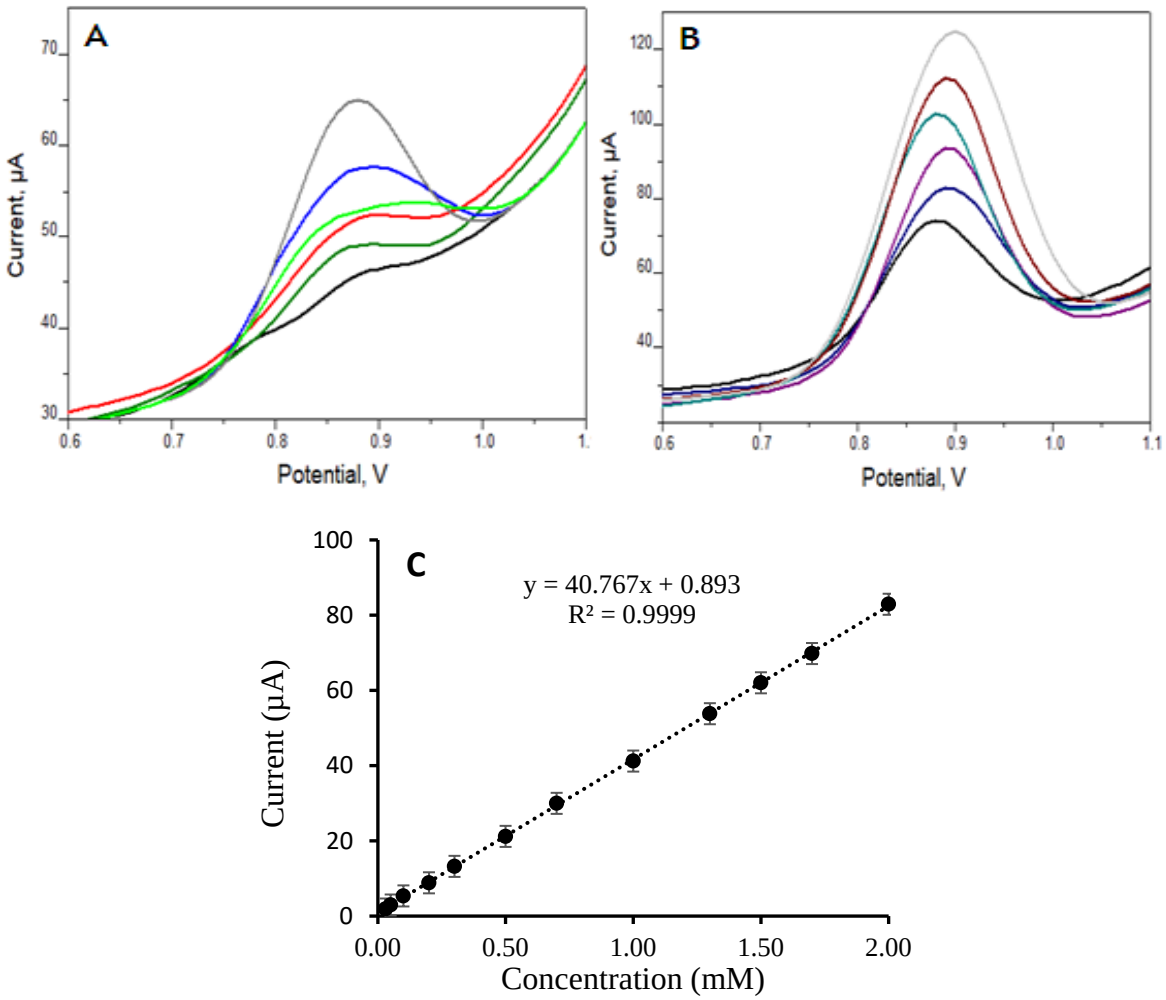


Fig. 10. Square wave voltammograms of low concentration **(A)**, high concentration **(B),** and α-lipoic acid calibration graph**(C)** in optimum conditions.

3.8. Analysis of real sample

The applicability of the developed method for health disease or environment need to be studied in real medium. Into this work, the modified electrode was employed in the detection of α-LA in synthetic urine samples. The recovery studies were performed by adding a known amount of α-LA in the samples as depicted in Table 1. According to the results obtained in the synthetic urine, it is clear that the invented electrode is adequate for real sample analysis (Table 1).

Table 1. Determination of α-LA synthetic urine samples (n=3)

Sample	Original found in sample (µM)	Standard added (µM)	Total Found (µM)	RSD (%)	R (%)
Synthetic urine	Not detected	500.00	467.20±0.05	4,82	93.44
Synthetic urine	Not detected	700.00	702.00±0.02	2.02	100.20

4. CONCLUSION

The research method presented here was carried out using simple, selective, sensitive, low cost, easily prepared Pt working electrodes that require only a short analysis time to determine α-LA using *poly*(VA). There is interest in the deposition of α-LA with a fast and easily prepared *poly*(VA) on platinum electrode in BR buffer (pH=5) electrolyte. The optimum conditions for determination of α-LA were as follows BR buffer (pH=5), 0.6 V accumulation potential, an accumulation time of 60 s and a pulse amplitude of 50 mV. The developed sensor is under optimum conditions; It showed sensitivity and selectivity towards α-LA with a linearity in the range of 0.03 mM to 2.0 mM. The quantitation limit and detection limit were 0.075 mM and 0.025 mM respectively after 60 s accumulation time. Furthermore, the applicability of the proposed electrode in synthetic urine samples gives promising results.

Conflicts of Interest

No conflict of interest was declared by the author.

REFERENCES

[1] G.P. Biewenga, G.R.M.M. Haenen, and A.A. Bast, Gen. Pharmacy 29 (1997) 315.

[2] V.D. Martins, V. Manfredini, M.C.R. Peralba, and M.S. Benfato, Clin. Nutr. 28 (2009) 192.

[3] A. Gorąca, H. Huk-Kolega, A. Piechota, P. Kleniewska, E. Ciejka, and B. Skibska, Pharm. Rep. 63 (4) (2011) 849.

[4] P. Arivazhagan, P. Juliet, and C. Panneerselvam, Pharmacol. Res. 41 (2000) 299.

[5] É. Kozlov, I. Solunina, M. Lyubareva, and M. Nadtochii, Pharm. Chem. J. 38 (2004) 642.

[6] K. Winiarska, D. Malinska, K. Szymansky, M. Dudziak, and J. Bryla, Biochimie 903 (2008) 450.

[7] A. Azimi, and M. Khalili, In: Probiotics, Prebiotics, and Synbiotics. Bioactive Foods in Health Promotion, R.R. Watson and V.R. Preedy (Ed.), Elsevier (2016) London, United Kingdom.

[8] M. Marin, C. Lete, N.B. Manolescu, and S. Lupu, J Electroanal. Chem, 729 (2014) 128.

[9] S. Sechovcová, P. Královcová, R. Kanďár, and K. Ventur, Biomed Chromatogr. 32 (2018) 4172.

[10] H.T. Chng, L.S. New, A.H. Neo, C.W. Goh, E.R. Browne, and E.C.Y. Chan, J Pharmaceut. Biomed. 51 (2010) 754.

[11] Z. Korijanac, M. Cakar, S. Tanaskovi, and T. Jovanovi, J. Serb. Chem. Soc. 72 (2007) 29.

[12] S. Satoh, M. Shindoh, J.Z. Min, T. Toyo'oka, T. Fukushima, and S. Inagaki, Anal. Chim. Acta 618 (2008) 210.
[13] B. Karakoyun, M. Yüksel, F. Ercan, C. Erzik, and B.Ç. Yeğen, Inflammation 32 (2009) 37.
[14] AI. Durrani, H. Schwartz, W. Schmid, and G. Sontag, J Pharm Biomed Anal. 45 (2007) 694.
[15] H. Li, Y. Kong, L. Chang, Z. Feng, N. Chang, J. Liu, and J. Long, Chromatographia 77 (2014) 145.
[16] L.N.S. Pereira, I.S. Silva, T.P. Araújo, A.A. Tanaka, and L. Angnes, Talanta 154 (2016) 249.
[17] S. Titretir, G. Erdogdu, and A. E. Karagözler, J Anal Chem. 61 (2006) 592.
[18] F. Karimian, G.H. Rounaghi, and M. Mohadeszadeh, J Anal Chem. 71 (2016) 1057.
[19] T. Rohani, and A. Ghaderi, J Anal Chem. 73 (2018) 277.
[20] M. Burç, Ö. Güngör, S. Titretir Duran, Anal. Bioanal. Electrochem. 12 (2020) 625.
[21] A. Paşahan, N. Ayhan, İ. Özcan, S. Titretir Duran, and S. Köytepe, Polym. Plast Technol Eng. 58 (2019) 1125.
[22] Ö. Güngör, İ. Özcan, M.A. Erdoğan, B. Ateş, and S. Köytepe, Analytical Letters 53 (2020) 228.
[23] S. Titretir Duran, J Turkish chem soc. 5 (2018) 1021.
[24] K. Ezhilarasi, V. Sudha, R. Geetha, U. Dhamodharan, R. Rama, P. Indira, V. Vijay, A.K. Hemanth Kumar, J Chromatograph Separat Techniq. 5 (2014) 1.
[25] R. Sasikumara, P. Ranganathanb, S-M. Chena, and S-P. Rwei, Sens. Actuators B, 255 (2018) 217.
[26] A. Skorupa, and S. Michalkiewicz, Int. J. Electrochem. Sci. 14 (2019) 5107.
[27] L. Mialon, A.G. Pemba, and S.A. Miller, (Communication) Green Chem. 12 (2010) 1704.
[28] M.B. Hocking, J. Chem. Educ. 74 (1997) 1055.
[29] G.S. Garbellini, R.C. Rocha-Filho, and O. Fatibello-Filho, Anal. Meth. 7 (2015) 3411.
[30] A.S. Amarasekara, B. Wiredu, and A. Razzaq, Green Chem. 14 (2012) 2395.
[31] J.G. Manjunatha, B.E. Kumara Swamy, M. Deraman, and G.P. Mamatha, Der Pharma Chemica 4 (2012) 2489.