



VOLTAMMETRIC BEHAVIOUR OF ENOXIL IN AQUEOUS MEDIUM OF NaClO₄ ON PLATINUM ELECTRODE

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The paper presents a study of the voltammetric behaviour of Enoxil on a platinum electrode in aqueous solution of 0.1M NaClO₄. On the basis of plotted cyclic voltammograms (CV) was found that the Enoxil undergoes a quasi-reversible redox process, which may be used for identification and dosage of this biologically active compound.

The values of the standard reduction and oxidation potentials and the formal redox potential of Enoxil were determined and by the rotating disk electrode technique (RDE) was followed the determining mechanism of redox process undergone by Enoxil in the considered environment.

Another aim of this research was to study the influence of different additions of ascorbic acid and oxalic acid, which facilitates the conjugated redox process, specific to the Enoxil, and the addition of hydrogen peroxide, which leads to the consumption of the Enoxil, the oxidizing action of the hydrogen peroxide is further manifested on this product that was obtained by grinding the grape seeds, extraction with ethanol and treatment with hydrogen peroxide.

INTRODUCTION

The Enoxil is a mixture of natural compounds obtained by the oxidation of tannins extracted from grape seeds. Its structure is not well known but the previous analysis (MS, IR spectroscopy, NMR) highlights the presence of flavanol subunits.

Being extracted from grape seeds, the Enoxil is considered a secondary winery product and it can be used for the protection of sugar beet, soybean and vines against the attack of fungal pathogens.^{1,2} This biologically active compound presents a strong antioxidant activity, having the ability to capture free radicals, this property being also pointed in the current paper through voltammetric method.

Condensed tannins, isolated from grape seeds, called proanthocyanidins, are a group of compounds with polyphenolic structure with biological activity, whose use in human diet is a preventive measure to

combat serious diseases like cancer or stroke. The tannins from grape seeds presents interest, primarily due to their antioxidant activities, knowing that the redox potential is low enough to work with various oxidizing species, most often by capturing free radicals. There are studies about the effect of enotannins on enzymatic systems such as phospholipases A₂, cyclooxygenase and lipooxygenases, which is another proof of the significance of enotannins to human health.^{3,4}

The proanthocyanidins isolation from grape seeds is rather a complicated problem, initially taking place the grinding of raw material, followed by the extraction with a suitable solvent, such as acetone, acetone-water, methanol, methanol with the addition of ascorbic acid followed by the mixture methanol-water and acetone-water at -24°C and acetone-water in an atmosphere of nitrogen, methanol-water with ultrasonic irradiation or ethanol.⁵⁻⁹

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The Enoxil was obtained by the oxidation of proanthocyanidins with hydrogen peroxide and presents an antioxidant activity with over 20% higher than the initial enotannins. The study of ascorbic acid, oxalic acid and hydrogen peroxide influence was made because this product is intended to be used in human diet so it is important to know how it interacts with other substances from human body.

RESULTS AND DISCUSSION

Cyclic voltammograms were drawn on the domain $-800 \div 1200 \div -800$ mV, noticing that the 0.1 M aqueous solution of NaClO_4 , on this potential field, does not present a significant peak, while by adding the Enoxil, the cyclic voltammograms present a cathodic peak (E_{pc}) coupled with an anodic peak (E_{pa}), that suggests the existence of a redox couple characteristic to Enoxil, allowing its identification by voltammetry. In Fig. 1 are represented the two peaks from the cyclic voltammograms at different scanning speeds, the equation of the linear dependence of peak current intensity on the square root of scanning speed (\sqrt{v}) being written in the figure.

Because the shift of the cathodic peak potentials to smaller values and the one of the anodic peak to higher values along with the increase of scanning speed is not significant, as it can be seen in Fig. 1, results that the redox process generated by the Enoxil tends towards reversibility. This property allows us to define a formal redox potential (E^0) for the redox couple shown, based on the two peak potentials.

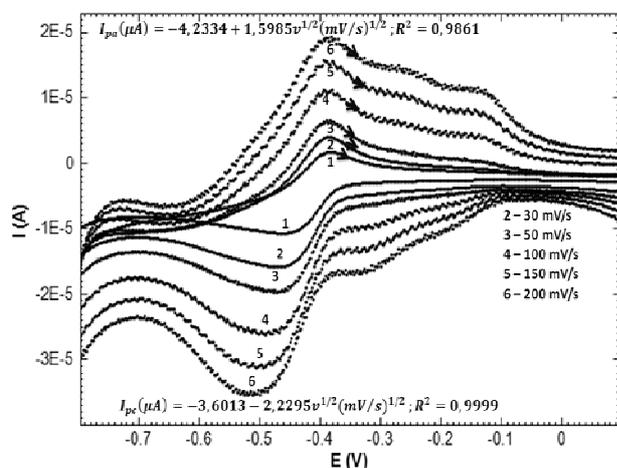


Fig. 1 – Characteristic peaks of Enoxil at concentration of 0.0404g/dL at different scanning speeds

$$E^0 = \frac{E_{pa} + E_{pc}}{2} \quad (1)$$

To highlight the determinant stage of the redox process undergone by Enoxil, was used the rotating disk electrode (RDE) technique, the scanning speed being 10mV/s and the rotation speed of RDE were 2, 8, 16, 20 and 25 rot/s.^{10,11}

If the Lewich criterion is verified, meaning that the dependency $I_{d,l} = f(\omega^{1/2})$ is linear, the determinant step of the electrode process is the diffusion, which in the case of Enoxil is checked only for the reduction peak that appears by the drawing of the polarization curve using RDE technique, on the domain $-100 \div -1000$ mV, this being illustrated in Fig. 2.

In the Fig. 3 are represented the CV at different concentrations of the Enoxil at a scanning speed of 20mV/s; for the cathodic process being possible to establish a linear dependency between I_{pc} and the concentration, which gives the possibility of quantitative determination of the Enoxil from solution, the equation being presented in the figure.

By plotting the cyclic voltammograms (Fig. 3, CV 6) for the Enoxil solution to which was added a natural syrup, it was highlighted that the electroactive species present in Enoxil can be also found in this commercial syrup obtained from grape seeds and skins, but could not be found by voltammetric method in other different commercial products obtained from grapes. In Fig. 3 it can be seen that by adding the natural syrup (CV 6) occurs an increase of peak intensities but the position of the peak potentials remain the same as in the case of the Enoxil.

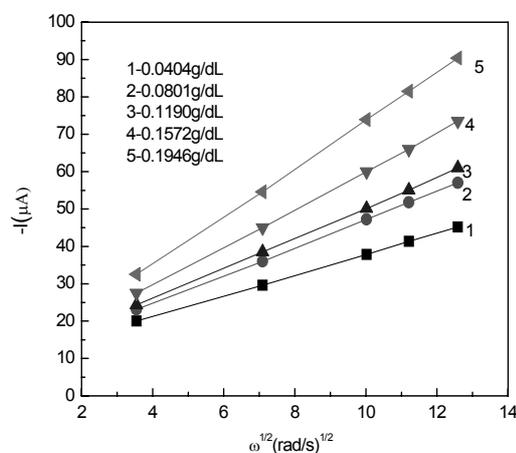


Fig. 2 – Verification of Lewich criterion for the reduction of Enoxil

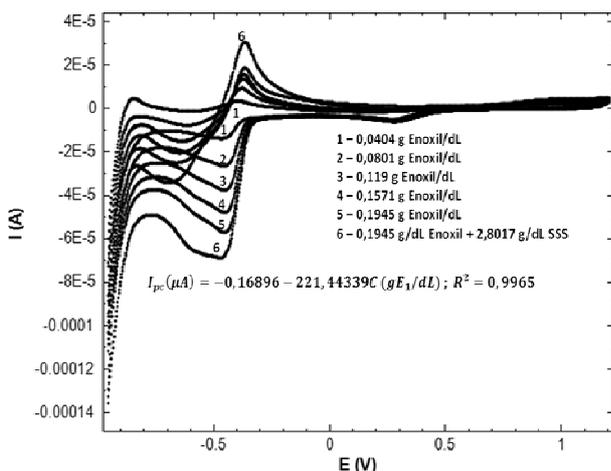


Fig. 3 – CV of aqueous Enoxil solutions at different concentration at a scanning speed of 20mV/s.

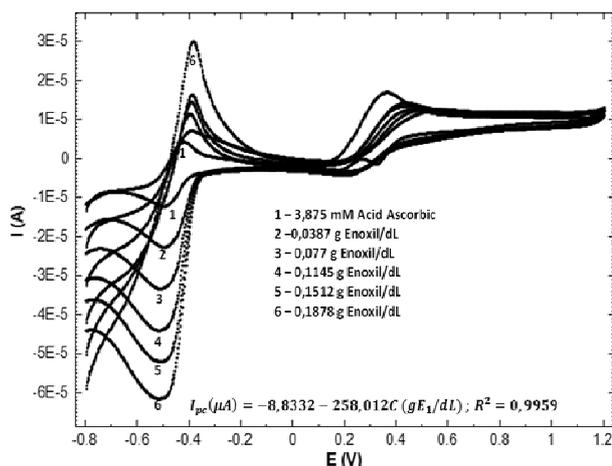


Fig. 4 – CV of ascorbic acid (3.875mM) in the presence of aqueous Enoxil solution at a scanning speed of 20mV/s.

In Fig. 4 are represented the cyclic voltammograms plotted for the solution of ascorbic acid (1) in 0.1M NaClO₄, which show that, besides the characteristic oxidation peak,¹² appears an anodic peak and a cathodic peak specific to the electroactive species from Enoxil (a slight shift of anodic potential towards positive values), and then by adding the Enoxil, the intensities of the two peaks grow more pronounced than in the absence of ascorbic acid.

Not the same thing happens by adding to the Enoxil solution, different concentrations of ascorbic acid. In this case there is an increase of peak intensities, but less pronounced. The increase of the ascorbic acid amount leads to a pronounced increase of the oxidation peak,¹² located at positive potential, as shown in Fig. 5.

The presence of the oxalic acid in 0.1M NaClO₄ solution, is not leading to any peak in the domain characteristic to the Enoxil (CV (1) from Fig. 6) while by adding the Enoxil samples it can be seen the anodic and the cathodic peak specific to the Enoxil (CV 2÷6 from Fig. 6) with higher values for the peak intensities than in the presence of ascorbic acid (Fig. 5).

Starting from the fact that the synthesis of the Enoxil contains a step in which the grape seeds extract is treated with hydrogen peroxide, in this research it was also studied the influence of hydrogen peroxide on the behavior of Enoxil, this being represented in the Fig. 7.

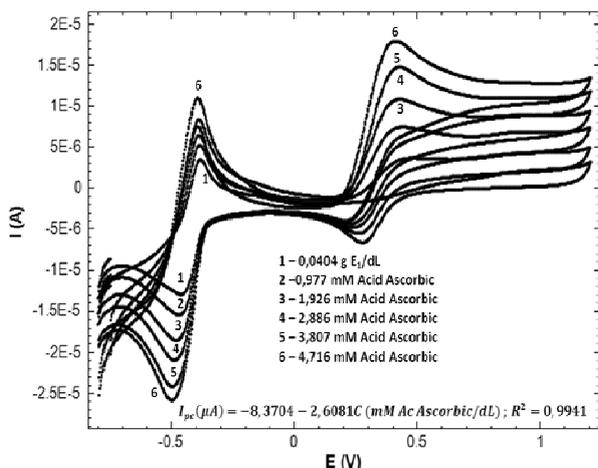


Fig. 5 – CV of aqueous solution of Enoxil (0.0404g/dL) at different concentrations of ascorbic acid at 20mV/s.

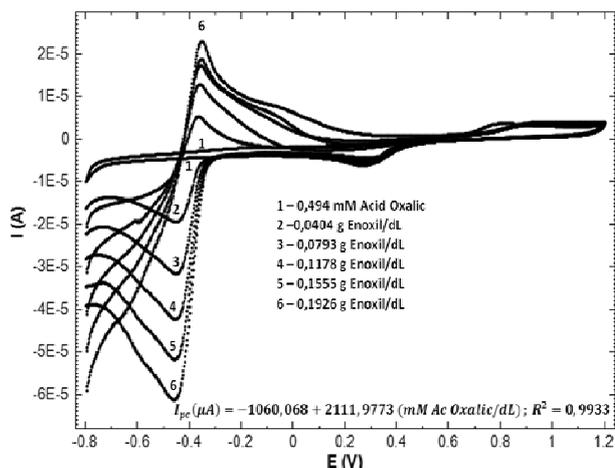


Fig. 6 – CV of oxalic acid (0.494mM) in the presence of increasing concentrations Enoxil at 20mV/s.

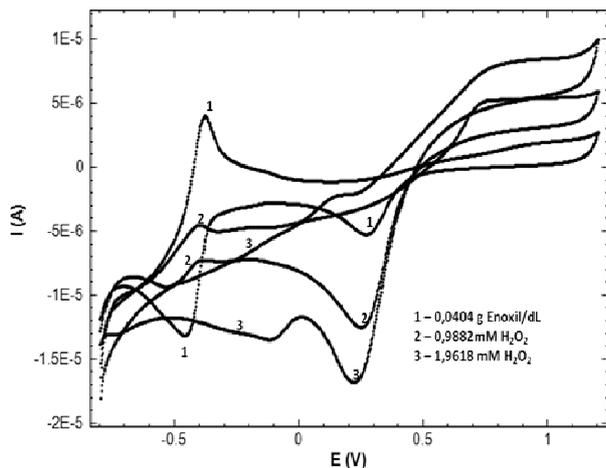


Fig. 7 – The influence of hydrogen peroxide on the voltammetric behavior of the Enoxil at a 20mV/s scanning speed.

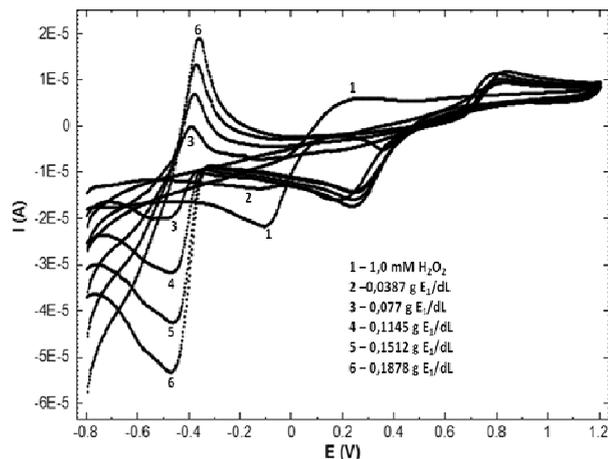


Fig. 8 – CV of aqueous solutions of Enoxil in the presence of hydrogen peroxide (1.0mM), at the scanning speed of 20mV/s.

Table 1

The voltammetric characteristics of the Enoxil solution and its mixtures, on Pt electrode, at 20mV/s and 25°C in 0.1M NaClO₄

Composition	-E _{pc} (mV)	-I _{pc} (μA)	-E _{pa} (mV)	I _{pa} (μA)	-E ^o (mV)
0,0404 g/dL Enoxil	464	8.77	368	4,69	416.0
0,1945 g/dL Enoxil	457	42.49	371	30.86	414.0
0,0404 g/dL Enoxil+1,922 mM Acid oxalic	472	26.98	348	23.56	410.0
0,494 mM Acid oxalic+0,1945 g/dL Enoxil	465	55.26	349	42.04	407.0
0,0404 g/dL Enoxil+0,977 mM Acid Ascorbic	469	10.91	370	10.67	419.5
0,0404 g/dL Enoxil+4,716 mM Acid Ascorbic	472	26.98	348	23.56	410.0
0,1945 g/dL Enoxil+2,8017g/dL SSS	474	68.39	371	30.94	422.5
1 mM H ₂ O ₂ +0,1945 g/dL Enoxil	467	53,04	367	18.87	417.0

In the second cyclic voltammogram from Fig. 7, the peak intensities (both anodic and cathodic) decrease, proof that the electroactive component from the Enoxil in the presence of H₂O₂ is consumed and the third cyclic voltammogram shows that this component has disappeared, along with a new addition of H₂O₂. Fig. 8 shows that the adding of the Enoxil in hydrogen peroxide solution (cyclic voltammograms (1) from Fig. 8), leads to the consumption of H₂O₂ and then the progress of the redox quasi-reversible process specific to the Enoxil (cyclic voltammograms (3÷6) from Fig. 8).

In Table 1 are given the characteristics of the solutions of maximum and minimum concentration of the Enoxil and also the mixtures whose voltammograms were presented, indicating that in the column where is written the composition, the components were written in the order in which they were added in the electrochemical cell that was containing 0.1M NaClO₄ solution.

The pH of the aqueous solution of Enoxil with the electrolytic background, 0.1M NaClO₄, is below 2.80 and by increasing the Enoxil

concentration, the pH decreases. The electrical conductivity of the studied solutions is sufficiently high, ranging in the domain 9.4÷11.0 mS/cm, these values of *k* give certainty that the ohmic loss of the analyzed solutions does not affect the form of the cyclic voltammograms.

The cathodic peak characteristic to the Enoxil appears in the range of -457÷-474 mV and the anodic one in the range of -348÷-371 mV, except when the hydrogen peroxide is in excess and the electroactive species is consuming, and the formal redox potential calculated with the equation (1) has the values in the domain -407.0÷-422 mV. The values of peak intensities show that the presence of the oxalic acid facilitates the quasi-reversible redox process of the Enoxil more than the presence of the ascorbic acid.

EXPERIMENTAL

For the plotting of cyclic voltammograms (CV) was used a cell with three electrodes: the working electrode (WE) – a platinum disk electrode (PtDE) with Φ=2mm placed in a

Teflon mantle, the auxiliary electrode (AE) – a plane electrode of platinum (10 x 20mm), and as reference electrode (RE) – saturated calomel electrode (SCE), connected to the Autolab PG STAT302N Potentiostat (Metrohm Autolab), which also has integrated the software Nova 1.6.¹¹

The rotating disk electrode technique was applied using Electrochemical Combine VoltaLab32 (Radiometer Copenhagen) equipped with the software VoltaMaster2 and with an electrochemical cell with three electrodes,¹²⁻¹⁴ the working electrode – PtDE ($\Phi=2\text{mm}$), the reference electrode – SCE and the auxiliary electrode – a platinum wire electrode ($\Phi=1\text{mm}$, $h=10\text{mm}$).

For the measurements of pH and electroconductivity was used an electrochemical multimeter Consort 861 (Belgium), the work temperature was 25°C, and the removal of oxygen from the system before any determination was made by bubbling N₂.

The studied Enoxil^{15,16} was dissolved in bidistilled deionized water, obtaining a solution with the concentration of 4.04g/L. The ascorbic acid, oxalic acid, NaClO₄ and 30% hydrogen peroxide were from the company Merck, and for the preparation of the solutions was also used bidistilled deionized water. Also, for comparison, was used a commercial syrup extracted from grape seeds (SSS), which was voltammetric characterized similar to Enoxil.

CONCLUSION

According to the voltammetric studies of aqueous solutions of 0.1M NaClO₄ on platinum electrode we can conclude that the Enoxil suffers a quasi-reversible redox process, located to sufficiently negative potential values, which highlights the antioxidant properties, allowing its identification by voltammetry.

Using the rotating disk electrode technique was demonstrated that the determinant step of the reduction process of the electroactive species is the diffusion, the Lewich criterion being verified and the slow transport of the electroactive species from the Enoxil solution owing to its size.

The additions of oxalic acid and ascorbic acid facilitate the quasi-reversible redox process, while the hydrogen peroxide interacts with the Enoxil.

It was established the dependency of the peak currents of the reduction process on the Enoxil concentration which allows its quantitative determination and was calculated the formal redox

potential of the quasi-reversible redox process of the Enoxil which can be used in qualitative estimations.

By plotting the cyclic voltammograms was observed that the electroactive component of the Enoxil which leads to the quasi-reversible redox process, was revealed also in a commercial syrup grape seeds.

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