

DEVELOPMENT OF CYCLIC VOLTAMMETRIC METHOD FOR THE STUDY OF THE INTERACTION OF ANTIOXIDANT STANDARDS WITH SUPEROXIDE ANION RADICALS CASE OF α -TOCOPHEROL

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Abstract: This work aims to develop a cyclic voltammetry based assay for the calculation of binding parameters like binding constant, ratio of binding constants, and binding free energy of the free and superoxide anion radical bound forms of α -tocopherol (TOC) antioxidant standard. The determination of binding constant is based upon the decrease in anodic peak current of the redox $O_2/O_2^{\cdot-}$ couple in the presence of antioxidant standard, whereas the mode of interaction is concluded from the shift in peak potential values. The diffusion coefficients of both free superoxide anion radical and its bounded forms with TOC were also evaluated from Randles-Sevcik equation.

Keywords: *binding constants, commercial oxygen, diffusion coefficients, free energy, Randles-Sevcik equation*

INTRODUCTION

Although thousands of papers have reported studies on the antioxidant activity of phenolic compounds and antioxidant standards using different methods based on scavenging activity of free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion ($O_2^{\circ-}$), information on binding parameters of these radicals with antioxidant standards or with potential antioxidant compounds have never been studied so far.

The aim of this work is to provide a method, based on cyclic voltammetry measurements, for the determination of binding parameters of the antioxidant standard α -tocopherol with superoxide anion radical. In addition, diffusion coefficients of the free and $O_2^{\circ-}$ bound forms of α -tocopherol were evaluated from Randles-Sevcik equation.

MATERIALS AND METHODS

Chemical

Dimethylformamide (DMF) (analytical grade from PROLABO) was used as solvent without further purification, tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (electrochemical grade (99 %) Sigma-Aldrich) was used as supporting electrolyte and its concentration was kept 0.1 M, α -tocopherol (97 %) from Alfa Aesar. All other reagents used are (analytical grade).

Instrumentation and software

Cyclic voltammetry measurements were performed using PGZ301 potentiostat (radiometer analytical SAS) and a voltammetric cell with a volumetric capacity of 50 mL containing a glassy carbon electrode (GC) working electrode (radiometer analytical SAS), a Pt wire counter electrode, and an Hg/Hg_2Cl_2 reference electrode (3.0 M KCl). Solutions were saturated with high purity commercial oxygen for 10 min prior to each experiment. Data acquisitions were accomplished with a Pentium IV (CPU 4.0 GHz and RAM 2 Gb) microcomputer using VoltaMaster4 software version 7.08 (radiometer analytical SAS). Graphs plot and calculus were carried out using OriginLab software version 2.0 (Integral Software, France).

RESULTS AND DISCUSSION

Voltammetric studies of TOC– $O_2^{\circ-}$ interaction

The decrease in anodic peak current of the redox $O_2/O_2^{\circ-}$ couple in the presence of α -tocopherol can be used for the calculation of the binding constant, whereas the shift in peak potential values can be exploited for the determination of the mode of interaction,

following the same methodology to that employed for studying the binding of drug molecules to DNA [1-4].

Commercial oxygen of high purity supplied from Lind-Gas Algérie was used to generate superoxide anion radicals by bubbling it in an electrochemical cell containing dimethylformamide as a solvent and tetrabutylammonium tetrafluoroborate as supporting electrolyte. Typical CV behavior of oxygen-saturated DMF/0.1 TBFP in the potential window of -0.2 to -1.6 V at a glassy carbon electrode in the absence and presence of 0.2 mL of 1 mM solution of α -tocopherol in the same solvent is shown in Figure 1.

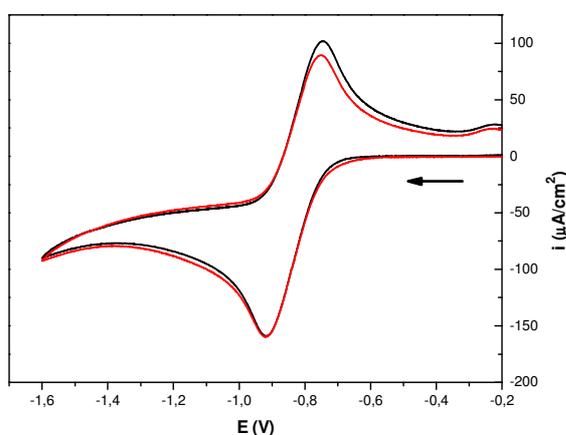
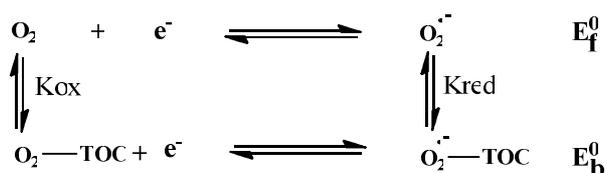


Figure 1. Cyclic voltammograms of oxygen-saturated DMF/0.1 TBFP on a polished GC electrode in the absence (black line) and presence of 0.2 mL (red line) of α -tocopherol

By adding 0.2 mL of α -tocopherol, the peak potential was shifted by ΔE_f^0 mV to more negative values associated with a significant decrease in anodic peak current $\Delta ip_a \%$ which confirm the interaction of α -tocopherol with $O_2^{\circ-}$, Table 1. The significant drop in anodic peak current can be assigned to the decrease in superoxide anion radical concentration due to the formation of slowly diffusing $O_2^{\circ-}$ -TOC product.

Ratio of binding constants (K_{ox}/K_{red})

The peak potential shift to more negative values additionally pointed out that O_2 is easier to oxidize in the presence of α -tocopherol because its reduced form $O_2^{\circ-}$ is more strongly bound to α -tocopherol than its oxidised form. For such a system, where both forms of the redox couple $O_2/O_2^{\circ-}$ interact with α -tocopherol antioxidant standard, Scheme 1 can be applied [5].



Scheme 1. General redox process of the free and $\text{O}_2^{\cdot-}$ bound TOC

Based upon the process presented in Scheme 1, the following equation is obtained [6],

$$E_b^0 - E_f^0 = 0.059 \log \frac{K_{ox}}{K_{red}} \quad (1)$$

Where E_f^0 and E_b^0 are the formal potentials of the $\text{O}_2/\text{O}_2^{\cdot-}$ couple in the free and bound forms respectively. Values of peak potential shift are summarised in Table 1 which indicates that interaction of the reduced form $\text{O}_2^{\cdot-}$ with α -tocopherol is 1.07 times stronger than the oxidized form O_2 .

Table 1. Electrochemical data of free and $\text{O}_2^{\cdot-}$ bound form of α -tocopherol

Compound	i_{p_a} [μA]	E_{p_a} [V]	E_{p_c} [V]	E^0 [V]	ΔE^0 [mV]	Δi_{p_a} %	K_{ox} / K_{red}
$\text{O}_2^{\cdot-}$	139.11	-0.7477	-0.9195	-0.8336	-	-	-
$\text{O}_2^{\cdot-}\text{-TOC}$	126,46	-0.7513	-0.9195	-0.8354	-1.8	9.1	0.93

Binding constant

The addition of 0.1 to 2.5 mL of α -tocopherol in DMF to commercial oxygen-saturated DMF/0.1 TBFP solution, shows a 5.56 to 33.77 % overall decrease in peak current, Figure 2. The substantial diminution in peak current can be attributed to the decrease in free superoxide anion radical concentration due to the formation of slowly diffusing, heavy molecular weight $\text{O}_2^{\cdot-}\text{-TOC}$ product.

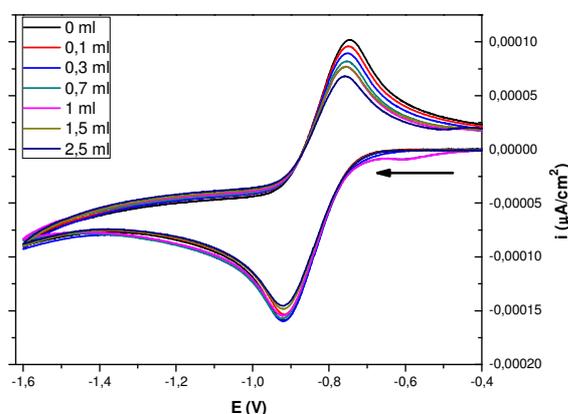


Figure 2. Cyclic voltammograms of oxygen-saturated DMF/0.1 TBFP on a polished GC electrode in the absence and presence of 0.1 mL, 0.3 mL, 0.7 mL, 1 mL, 1.5 mL and 2.5 mL α -tocopherol

The gradual decay in peak current of $O_2/O_2^{\circ-}$ couple by increasing of α -tocopherol concentration, ranging from 0.1 to 2.5 mL can be used to quantify the binding constant by the application of the following equation (2) [7],

$$\log \frac{1}{[TOC]} = \log K + \log \frac{1}{i_0 - i} \quad (2)$$

where $[TOC]$ is the concentration of α -tocopherol ($\text{mol}\cdot\text{L}^{-1}$), K is the binding constant (mol^{-1}), i and i_0 are the anodic peak current densities in the absence and presence of α -tocopherol. Figure 3 shows the plot of $\log 1/(1/[TOC])$ versus $\log 1/(i_0 - i)$.

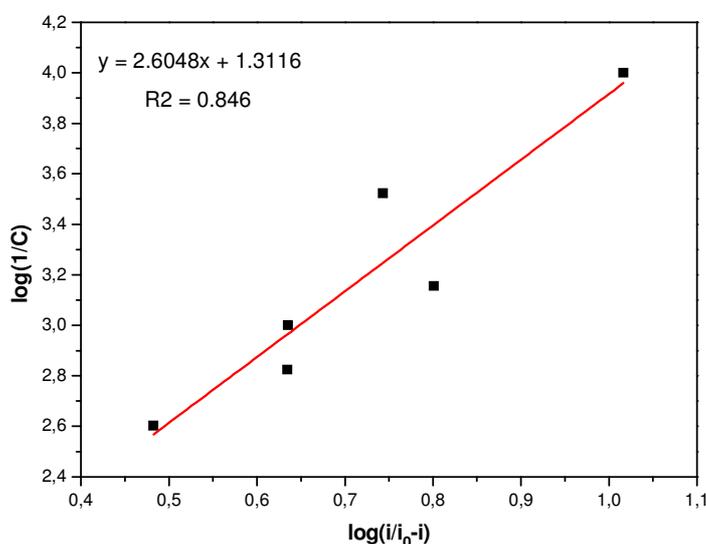


Figure 3. $\log 1/(1/[TOC])$ versus $\log 1/(i_0 - i)$ for $O_2^{\circ-}$ with varying concentration of α -tocopherol ranging from 0.1 to 2.5 mL in oxygen-saturated DMF/0.1 TBFP, used to calculate the binding constant of $O_2^{\circ-}$ -TOC product

Binding constant and binding free energy of studied α -tocopherol as obtained from the plot of equation 2 are listed in Table 2.

Table 2. Binding constants and binding free energy values

Compound	Equation	R^2	K [$\text{L}\cdot\text{mol}^{-1}$]	ΔG [$\text{KJ}\cdot\text{mol}^{-1}$]
$O_2^{\circ-}$ -TOC	$y=2.6048x+1.3116$	0.846	0.2×10^2	-7.41

The negative values of ΔG indicated the spontaneity of the $O_2^{\circ-}$ -TOC interaction.

Diffusion coefficients

The electrochemical behavior of free $O_2^{\circ-}$ radical and $O_2^{\circ-}$ -TOC at various scan rates is shown in Figure 3. Both voltammograms contained a couple of a well-defined stable

redox peaks attributed to the redox process of $O_2/O_2^{\circ-}$ couple. Figure 3 further reflects the reversibility of the redox processes as the peak potentials are not significantly affected by the variation in the scan rate.

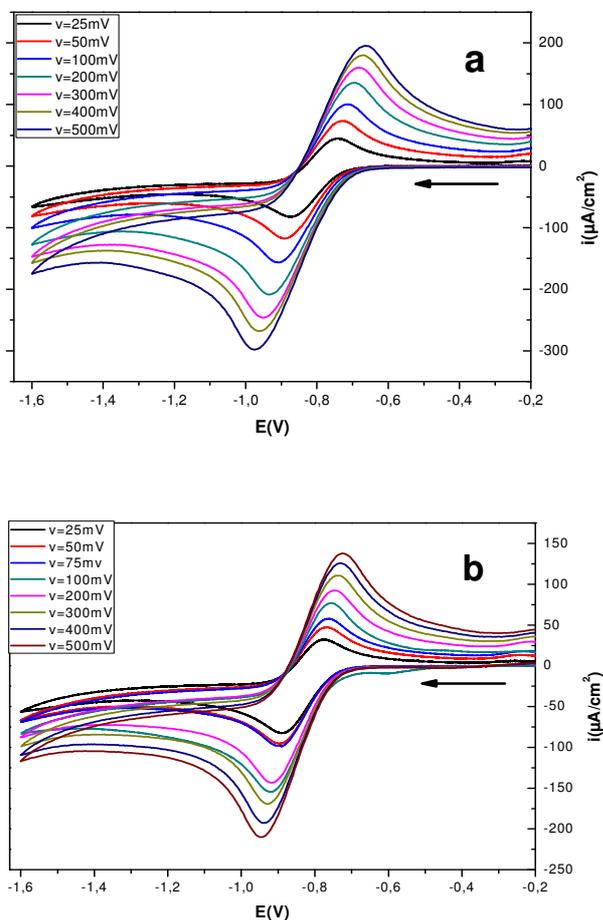


Figure 3. Succession of cyclic voltammograms at GC electrode of free $O_2^{\circ-}$ (a) and 0.1 mL of $O_2^{\circ-}$ –TOC (b) in oxygen-saturated DMF/0.1 TBFP at various scan rates

The diffusion coefficients of the free and $O_2^{\circ-}$ bound forms of TOC were determined using the following Randles–Sevcik equation [8].

$$i = 2.69 \times 10^5 n^{\frac{3}{2}} A C D^{\frac{1}{2}} v^{\frac{1}{2}} \quad (3)$$

where i is the peak current (A), A is the surface area of the electrode (cm^2), C is the bulk concentration ($mol \cdot cm^{-3}$) of the electroactive species, D is the diffusion coefficient ($cm^2 \cdot s^{-1}$) and v is the scan rate ($V \cdot s^{-1}$).

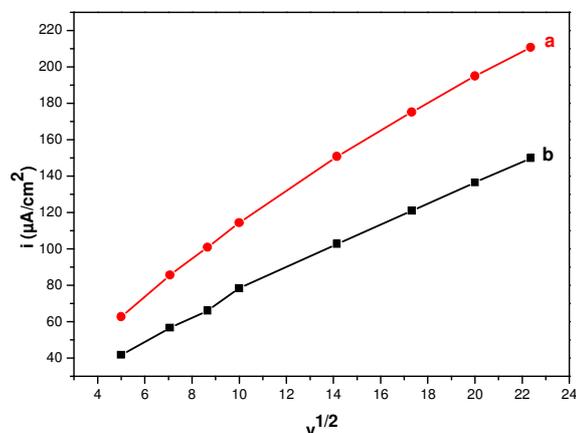


Figure 4. i_{p_a} vs. $v^{1/2}$ plots of oxygen-saturated DMF (a) in the presence of 0.1 mL of TOC (b) at different scan rates under the experimental conditions of Figure.3

The linearity of the relation $i_{p_a} = f(v^{1/2})$ for $O_2^{\circ-}$ -TOC suggests that the redox process is kinetically controlled by the diffusion step. The diffusion coefficients were determined from the slopes of Randles–Sevcik plots. Values are given in Table 3.

It can be seen that the diffusion coefficient of $O_2^{\circ-}$ bound TOC is remarkably lower than that of the free $O_2^{\circ-}$ radical.

Table 3. Diffusion constants values of free and $O_2^{\circ-}$ bound TOC

Compound	Equation	R ² values	D [cm ² ·s ⁻¹]
$O_2^{\circ-}$	$y = 2.4659 \times 10^{-4}x + 3.8412 \times 10^{-5}$	0.996	6.08×10^{-8}
$O_2^{\circ-}$ -TOC	$y = 1.8297 \times 10^{-4}x + 2.0637 \times 10^{-5}$	0.999	3.35×10^{-8}

The lower diffusion coefficient of $O_2^{\circ-}$ -TOC than $O_2^{\circ-}$ can be attributed to large molecular weight of the product $O_2^{\circ-}$ -TOC.

CONCLUSION

The binding parameters like binding constant, ratio of binding constants and binding free energy of antioxidant standard α -tocopherol with superoxide anion radical ($O_2^{\circ-}$), were successfully determined using cyclic voltammetry techniques. The results indicated electrostatic interaction of superoxide anion radical with antioxidant standard α -tocopherol as the dominant mode. The diffusion coefficients of the free and $O_2^{\circ-}$ bound forms of α -tocopherol were evaluated from Randles-Sevcik equation.

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