

**MESO-TETRA-p-TOLYLPORPHYRIN: SYNTHESIS, CHARACTERISATION,  
ANTIOXIDANT ACTIVITY AND DETERMINATION OF BINDING PARAMETERS  
OF WITH SUPEROXIDE ANION RADICAL**

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**ABSTRACT**

In this paper we present the synthesis and the evaluation of the antioxidant activity of meso-tetra-p-tolylporphyrin (TPPH<sub>2</sub>(p-methyl)) using superoxide anion radical (O<sub>2</sub><sup>-</sup>) in DMF as a solvent. The binding parameters of the interaction between the synthesised compound and the radical O<sub>2</sub><sup>-</sup> were also measured using cyclic voltammetry techniques. TPPH<sub>2</sub>(p-methyl) shows lower antioxidant activity (30.7 ± 0.08 mg/mL) than that of α-tocopherol standard (3.04 ± 0.03 mg/mL). The value of the binding Gibbs energy of -8.15 KJ.mol<sup>-1</sup> calculated for TPPH<sub>2</sub>(p-methyl) suggests an electrostatic interaction of O<sub>2</sub><sup>-</sup> with TPPH<sub>2</sub>(p-methyl) which has been found to be the dominant interaction mode. The kinetics of the interaction reaction was also measured having second-order rate constant values equal to 0.2 M<sup>-1</sup> s<sup>-1</sup>.

**Keywords:** Superoxide anion radical; antioxidant activity; cyclic voltammetry; porphyrin; binding parameters.

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## 1. INTRODUCTION

The stable superoxide anion free radical ( $O_2^-$ ) electrochemically generated by one-electron reduction of molecular oxygen in aprotic solvents is of great significance as a radical species implicated in diverse chemical and biological systems.

Superoxide anion free radical is used for the evaluation of the antioxidant activity as free radical  $O_2^-$  scavenging activity [1,2]. The assay is based on measuring the decrease in the anodic peak current density of the redox couple  $O_2/O_2^-$  upon addition of potentially antioxidant compounds. This decrease can also be used to determinate the binding parameters of the interaction of free radical  $O_2^-$  with potentially antioxidant compounds [3,4].

Meso-tetra-p-tolylporphyrin is a macrocyclic aromatic compound with  $18\pi$  electron structure, formed by four subunits of pyrrole linked together via methine bridges. Nitrogen atoms of meso-tetra-4-actophenyl-porphyrin can react with metal ions to produce very stable metalloporphyrins complexes, the most known metal ions complexes of meso-tetra-4-actophenyl-porphyrin are that of transition metal ions [5]. In the present study we report the determination of binding parameters of  $O_2^-$  free radical with the ligand TPPH<sub>2</sub>(p-methyl). The method is based on cyclic voltammetry measurements.

## 2. EXPERIMENTAL

### 2.1. Materials and methods

#### 2.1.1. Chemical

N,N-Dimethylformamide (DMF) (HPLC-grade from Sigma-Aldrich) was used as solvent in electrochemical assays, tetrabutylammonium tetrafluoroborate ( $Bu_4NBF_4$ ) (electrochemical grade (99%) Sigma-Aldrich) was used as supporting electrolyte and its concentration was kept 0.1 M, molecular oxygen is provided from a cylinder (research grade (99.99%) from Linde gaz Algérie),  $\alpha$ -tocopherol ( $C_{29}H_{50}O_2$ , MW = 430.71) (97 %), were all purchased from Alfa Aesar and used without further purification.

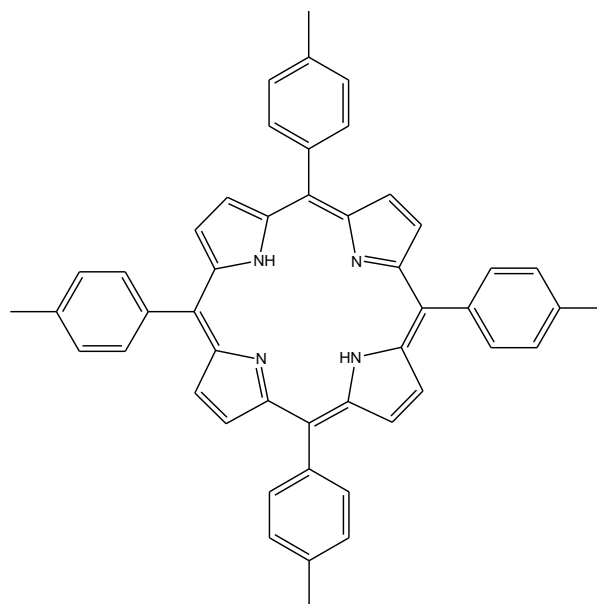
#### 2.1.2. Synthesis

The TPPH<sub>2</sub>(p-methyl) was synthesized from the reaction of 4-methylbenzaldehyde and

pyrrole in propionic acid (figure 1). 4-methylbenzaldehyde (35.3 mL, 340 mmol) was added to a well stirred solution of propionic acid (500 mL) and the colorless mixture was stirred at room temperature until all the aldehyde was completely dissolved, and then it was heat at 50°C. A solution of distilled pyrrole (25 mL, 360 mmol) in propionic acid (30 mL) was then added dropwise in about 10 minutes and the reaction mixture was refluxed for 30 minutes. It was then allowed to cool to room temperature, the resulting dark suspension was filtered, washed with 50 mL methanol/water (1:1) and then with methanol until the filtrate became clear. The obtained purple solid was dried in vacuum to yield 10.6 g of meso-tetra4-methophenyl-porphyrin (8.2%).

max (CH<sub>2</sub>Cl<sub>2</sub>)/nm 417, 514 (log M 5.92 and 4.23).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 300 K): δ = 8.88 (s, 8H, H<sub>β</sub>), 8.26 (s, 8H, H<sub>α</sub>), 7.79 (s, 12H, H<sub>m</sub>+H<sub>p</sub>), 9.1 (m, 12H) -2.70 (s, NH).



**Fig.1.** Structure of TPPH<sub>2</sub>(p-methyl)

### 2.1.3. Instrumentation and software

Cyclic voltammetry experiments were performed using PGZ301 potentiostat (radiometer analytical SAS) and a voltammetric cell with a volumetric capacity of 25 mL containing three electrodes, a glassy carbon working electrode, having area equal to 0.013 cm<sup>2</sup> a Pt wire counter electrode, and an Hg/Hg<sub>2</sub>Cl<sub>2</sub> reference electrode (3.0 M KCl). Solutions were

saturated with high purity commercial oxygen for 15 min prior to each experiment.  $^1\text{H}$  NMR spectra was collected on an Avance Bruker 400 spectrometer at 400.155 MHz spectrometer with deuterated chloroform at 7.26 ppm as an internal reference.

#### 2.1.4. Scavenging activity of superoxide anion radicals

The ability of the test sample to quench  $\text{O}_2^-$  radicals (% Inhibition of  $\text{O}_2^-$ ) was determined from the following equation (1) [6-8],

$$\% \text{ O}_2^- \text{ radical scavenging activity} = \frac{i_0 - i}{i_0} \times 100 \quad (1)$$

Where  $i_0$  and  $i$  are the anodic peak current densities of the superoxide anion radical in the absence and in presence of test potentially antioxidant compound.

### 3. RESULTS AND DISCUSSION

#### 3.1. Free radical scavenging activities study

In order to calculate the  $\text{IC}_{50}$  values,  $\text{O}_2^-$  radical scavenging activity was plotted against different compounds concentration (0.134, 0.268, 0.536, 0.737, and 1.34 mg/mL). The antioxidant capacity was expressed as  $\text{IC}_{50}$ . The equations obtained from the linear calibration graph in the studied concentration range for TPPH<sub>2</sub>(p-methyl) and  $\alpha$ -tocopherol are summarized in table 1 (where  $y$  represents the value of the anodic peak current density of  $\text{O}_2^-$  and  $x$ , the value of samples concentration, expressed as mg/mL). The antioxidant activity of TPPH<sub>2</sub>(p-methyl) is lower than that of  $\alpha$ -tocopherol standard.

#### 3.2. Antioxidant activity coefficient

The antioxidant activity coefficient ( $K_{\text{aac}}$ ) can be defined as the ratio of the pick of the anodic current density values of  $\text{O}_2^-$  in the presence and absence of the studied compound. This coefficient also measures the relative superoxide scavenging activity [9,10] and is calculated using the following equation 2,

$$K_{\text{aac}} = \frac{\Delta i}{(i_0 - i_{\text{res}})} \frac{1}{\Delta C} \quad (2)$$

where  $\Delta i$  is the variation in anodic pick current density caused by the addition of the antioxidant compound,  $i_0$  is the anodic pick current density in the absence of the studied antioxidant compound,  $i_{res}$  is the residual current density of oxygen in the system and  $\Delta C$  is the variation of the concentration of the studied antioxidant compound in mol/L.

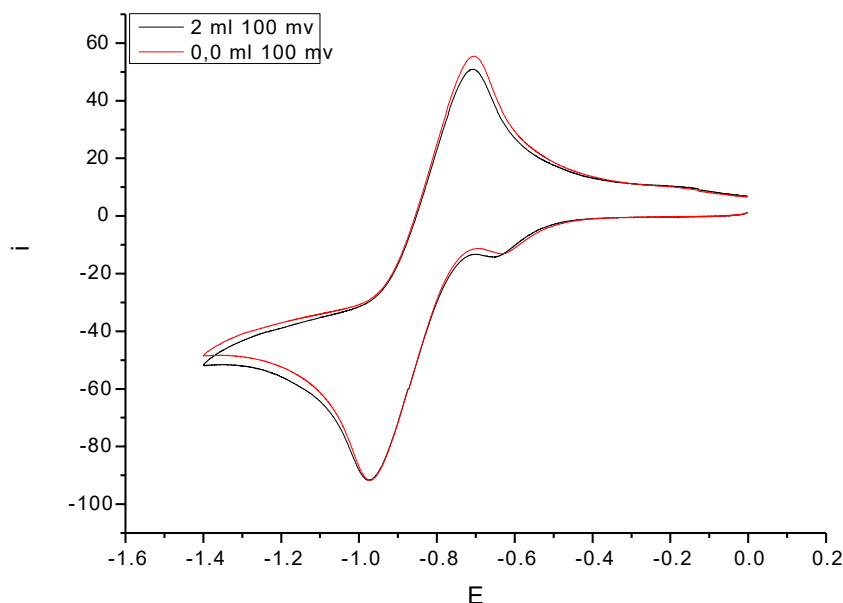
Equation 2 is only applied at low concentration of the studied antioxidant compound, i.e. for the linear change region. Obtained values of  $K_{aac}$  are shown below in table 1.

**Table 1.**  $IC_{50}$  values obtained using  $O_2^-$  radicals scavenging activity

Compound	Equation	$R^2$ values	$IC_{50}$ (mg/mL)	$K_{aac}$
TPPH <sub>2</sub> (p-methyl)	$y = 1.44411x + 5.6663$	0.947	$30.7 \pm 0.08$	$102.11 \pm 1.862$
$\alpha$ -tocopherol	$y = 15.990x + 1.3746$	0.950	$3.04 \pm 0.03$	$224.62 \pm 2.224$

### 3.3. Voltammetric studies of $O_2^-$ – TPPH<sub>2</sub>(p – methyl) interaction

The CV behavior of  $O_2^-$  in DMF/0.1M Bu<sub>4</sub>NBF<sub>4</sub> in the potential window of 0.0 to -1.4 V at a glassy carbon electrode in the absence and presence of a solution of 2 mM of TPPH<sub>2</sub>(p-methyl) in the same solvent is shown in figure 2. The free  $O_2/O_2^-$  redox couple exhibits one oxidation peak at 0.707 V and one reduction peak at 0.977 V. Figure 2 also shows the effect of the addition of TPPH<sub>2</sub>(p-methyl) to a solution of  $O_2^-$  in DMF/0.1M Bu<sub>4</sub>NBF<sub>4</sub> on the oxidation peak current density of  $O_2/O_2^-$  couple. The decrease in the anodic peak current density, caused by the addition of TPPH<sub>2</sub>(p-methyl), can be explained by the reaction of  $O_2^-$  with TPPH<sub>2</sub>(p-methyl) [11-13].

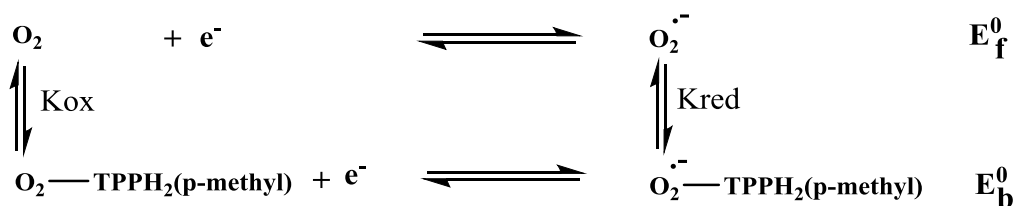


**Fig.2.** Cyclic voltammograms of oxygen-saturated DMF/0.1 Bu<sub>4</sub>NBF<sub>4</sub> on a GC electrode in the absence (black line) and in presence of (red line) 2 mM of TPPH<sub>2</sub>(p-methyl, scan rate 100 mV.s<sup>-1</sup> at T = 28° C

The addition of an amount of 2 mM of TPPH<sub>2</sub>(p-methyl) has caused a small shift in peak potential  $\Delta E^0$  in the negative direction associated with a decrease in anodic peak current density. Table 2 summarizes the obtained results; the significant drop in anodic peak current density can be assigned to the decrease in O<sub>2</sub><sup>-</sup> radical concentration due to the formation of O<sub>2</sub><sup>-</sup> – TPPH<sub>2</sub>(p-methyl) complex.

### 3.3.1. Ratio of binding constants ( $K_{ox}/K_{red}$ )

The peak potential shift of the O<sub>2</sub> / O<sub>2</sub><sup>-</sup> redox couple in the negative direction in the presence of TPPH<sub>2</sub>(p-methyl) indicates that the oxidation of O<sub>2</sub><sup>-</sup> is easier in presence of TPPH<sub>2</sub>(p-methyl) because its oxidized form O<sub>2</sub> is more strongly attached to TPPH<sub>2</sub>(p-methyl) than its reduced form O<sub>2</sub><sup>-</sup>. For such a system, where both forms of the O<sub>2</sub> / O<sub>2</sub><sup>-</sup> redox couple interact with TAcPPH<sub>2</sub> and PdTAcPP, scheme 1 can be applied [14].



**Scheme 1.** Redox process of the free and TPPH<sub>2</sub>(p-methyl) bound O<sub>2</sub><sup>•-</sup> redox couple

The application of the Nernst relation to the process presented in scheme 1 leads to equation (3) [15],

$$\Delta E^0 = E_b^0 - E_f^0 = E^0(\text{O}_2^{\cdot-} - \text{TAcPP}) - E^0(\text{O}_2^{\cdot-}) = 0.059 \log \frac{K_{\text{ox}}}{K_{\text{red}}} \quad (3)$$

$E_f^0$  and  $E_b^0$  are the formal potentials of the O<sub>2</sub>/O<sub>2</sub><sup>•-</sup> couple in the free and bound forms respectively. The decreasing rate of the anodic peak current density  $\Delta ip_a\%$  and the peak potential shift  $\Delta E^0$  are summarized in table 2.

**Table 2.** Electrochemical data of free and O<sub>2</sub><sup>•-</sup> bound form of TPPH<sub>2</sub>(p-methyl) used to calculate ratio of binding constants

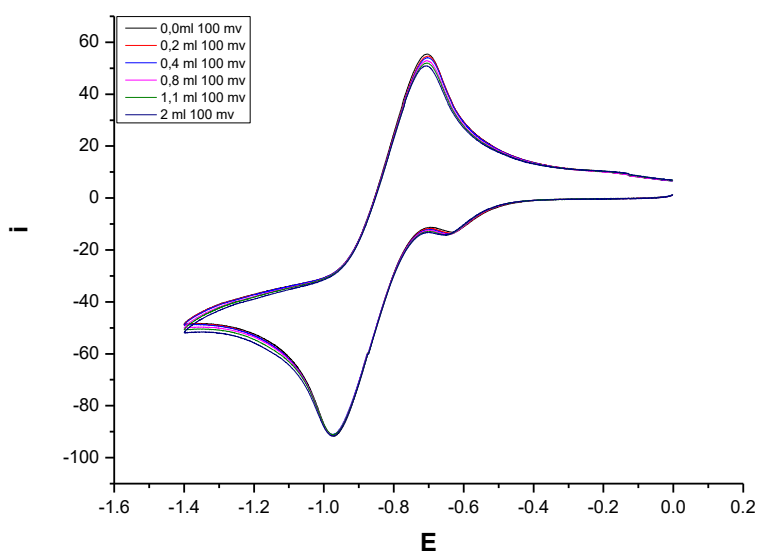
Compound	$Ep_a$ (V)	$Ep_c$ (V)	$E^0$ (V)	$\Delta E^0$ (mV)	$K_{\text{ox}} / K_{\text{red}}$
O <sub>2</sub> <sup>•-</sup>	-0.706	-0.973	-0.84	-	-
O <sub>2</sub> <sup>•-</sup> - TPPH <sub>2</sub> (p-methyl)	-0.706	-0.973	-0.84	00	1

The Ratio of the binding constants is calculated by replacing  $\Delta E^0$  from table 2 in equation 3. The obtained ratios of the binding constants indicate that the interaction of the reduced form O<sub>2</sub><sup>•-</sup> with TPPH<sub>2</sub>(p-methyl) is 1 times higher than its oxidized form O<sub>2</sub>.

### 3.3.2. Binding constant

The addition of increasing concentrations of TPPH<sub>2</sub>(p-methyl) in DMF to a solution of DMF saturated with commercial oxygen provokes remarkable decrease in the peak current density, (figure 3). The substantial decrease in anodic peak current density is due to the decrease in

$O_2^-$  concentration due to the formation of  $O_2^- - TPPH_2$  (p-methyl).



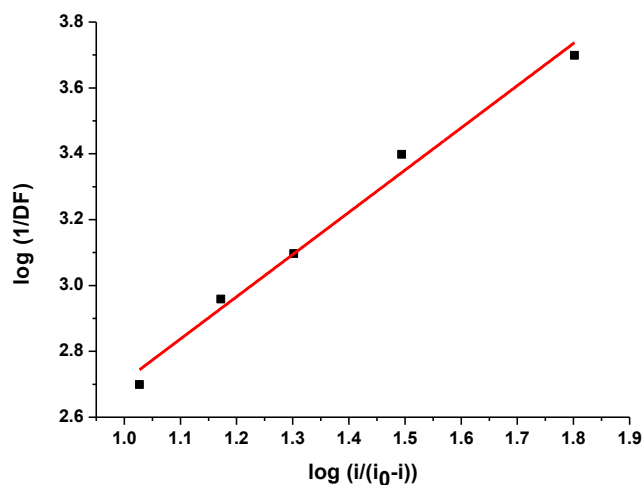
**Fig.3.** Cyclic voltammograms of oxygen-saturated DMF/0.1  $Bu_4NBF_4$  on a GC electrode in the absence and presence of different concentrations of TPPH<sub>2</sub>(p-methyl), scan rate 100 mV/s, at T = 28° C

The gradual decrease in peak current density of the  $O_2/O_2^-$  redox couple by increasing TPPH<sub>2</sub>(p-methyl) concentrations can be exploited to calculate the binding constant by applying the following equation (4) [15],

$$\log \left[ \frac{1}{[TPPH_2(p-methyl)]} \right] = \log K_b + \log \frac{i}{i_0 - i} \quad (4)$$

Figure 3 shows the plot of  $\log(1/C)$  versus  $\log i/(i_0 - i)$ .





**Fig.3.**  $\log(1/C)$  versus  $\log i/(i_0 - i)$  for  $O_2^-$  with varying concentration of TPPH<sub>2</sub>(p-methyl) in DMF/0.1 Bu<sub>4</sub>NBF<sub>4</sub>, used to calculate the binding constants of  $O_2^- - \text{TPPH}_2(\text{p-methyl})$  product

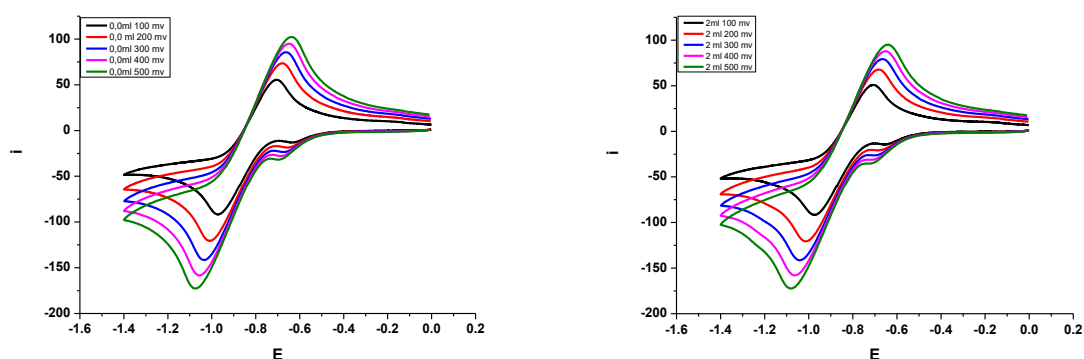
The intercept of the linear fitting of the plot  $\log(1/C)$  versus  $\log i/(i_0 - i)$  yielded the binding constants, from which the binding free energy was calculated, (table 3).

**Table 3.** Binding constants and binding free energies values of  $O_2^- - \text{TPPH}_2(\text{p-methyl})$  product

Compound	Equation	R <sup>2</sup>	K <sub>b</sub> (L.mol <sup>-1</sup> )	-ΔG (KJ.mol <sup>-1</sup> )
$O_2^- - \text{TPPH}_2(\text{p-methyl})$	$y = 1.28201 x + 1.42748$	0.984	26.76	8.15
α-tocopherol	$y = 0.841x + 2.298$	0.986	198.6	13.11

### 3.3.3. Diffusion coefficients

The diffusion coefficients of the free radical  $O_2^-$  and its bounded form  $O_2^- - \text{TPPH}_2(\text{p-methyl})$  were obtained by varying the potential scan rates (figure 4).

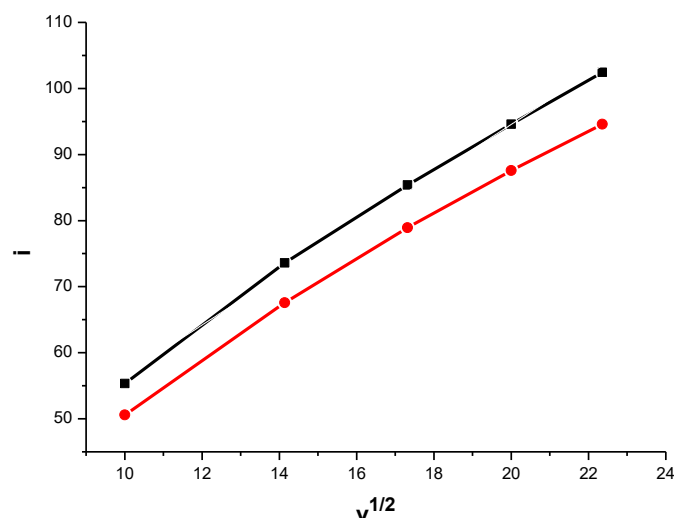


**Fig.4.** Succession of cyclic voltammograms at GC electrode of free radical  $O_2^-$  (a) and 2 mM of  $O_2^- - TPPH_2$  (p-methyl) (b) in oxygen-saturated DMF/0.1  $Bu_4NBF_4$  at various scan rates (100-500 mV, increment 100 mV) at  $T = 28^\circ C$ . The vertical arrowhead indicates increasing scan rate

To further confirm the interaction of  $O_2^-$  radicals with  $TPPH_2$ (p-methyl), the relation  $ip_a = f(\sqrt{v})$  was plotted before and after the addition of  $TPPH_2$ (p-methyl), using the following equation 5.

$$i = 2.69 \times 10^5 (\sqrt{n})^3 SC \sqrt{D} \sqrt{v} \quad (5)$$

The linear dependence of the peak currents density of both  $O_2^-$  and bounded forms  $O_2^- - TPPH_2$  (p-methyl) on the square root of the potential scan rates suggests that the redox process is kinetically controlled by the diffusion step, (figure 5).



**Fig.5.**  $i_{p_a}$  vs.  $\sqrt{v}$  plots of oxygen-saturated DMF/0.1 Bu<sub>4</sub>NBF<sub>4</sub> (a) in the presence of 2 mM of TPPH<sub>2</sub>(p-methyl) (b), at different scan rates under the experimental conditions of figure 4

The diffusion coefficients of the free and O<sub>2</sub><sup>-</sup> bound TPPH<sub>2</sub>(p-methyl) were determined from the slopes of Randles-Sevcik plots, values are summarized in table 4.

**Table 4.** Diffusion coefficients values of free and O<sub>2</sub><sup>-</sup> bound TPPH<sub>2</sub>(p-methyl)

Compound	Equation	R <sup>2</sup> values	D (cm <sup>2</sup> ·s <sup>-1</sup> )
O <sub>2</sub> <sup>-</sup>	$y = 3.79515x + 18.65192$	0.995	$1.64 \times 10^{-4}$
O <sub>2</sub> <sup>-</sup> – TPPH <sub>2</sub> (p – methyl)	$y = 3.55983x + 16.16603$	0.994	$1.44 \times 10^{-4}$

The diffusion coefficient of bounded O<sub>2</sub><sup>-</sup> – TPPH<sub>2</sub> (p – methyl) is small when compared to free O<sub>2</sub><sup>-</sup> is suggestive of O<sub>2</sub><sup>-</sup> – TPPH<sub>2</sub> (p – methyl) product formation. The reason of the decrease in the diffusion coefficient of O<sub>2</sub><sup>-</sup> in presence of TPPH<sub>2</sub>(p-methyl) is due to the higher molecular weight of the formed products.

#### 4. CONCLUSION

In the present work, we studied the interaction of superoxide anion radical with Meso-tetra-p-tolylporphyrin (TPPH<sub>2</sub>(p-methyl) using cyclic voltametry method. The decrease in anodic peak current density was used to evaluate the binding parameters of the interaction of O<sub>2</sub><sup>-</sup> with TPPH<sub>2</sub>(p-methyl. This study showed that the radical O<sub>2</sub><sup>-</sup> strongly interacts with TPPH<sub>2</sub>(p-methyl. Anodic peak current density of the O<sub>2</sub>/O<sub>2</sub><sup>-</sup> redox couple decreased as TPPH<sub>2</sub>(p-methyl amount was gradually increased in solutions containing a fixed concentration of O<sub>2</sub><sup>-</sup> radicals. The IC<sub>50</sub> of the ligand TPPH<sub>2</sub>(p-methyl is higher than α-tocopherol. The low diffusion coefficient of the bounded O<sub>2</sub><sup>-</sup> – TPPH<sub>2</sub>(p-methyl compared to that of the free O<sub>2</sub><sup>-</sup> evidenced the formation of product which diffuses slowly than free O<sub>2</sub><sup>-</sup> radical.

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