



Evaluation of total antioxidant capacity and free radicals scavenging of 2 and 4- Nitrophenylferrocene by electrochemical and chemical assays

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ABSTRACT

This work aims to investigate the *in vitro* antioxidant activity of two ferrocene derivatives: 2-nitrophenylferrocene and 4-nitrophenylferrocene using different assays, including DPPH radical scavenging assay, phosphomolybdenum reduction assay and superoxide anion radical. The results indicated that 2-nitrophenylferrocene has the most important percentage of scavenging DPPH radical with $IC_{50} = 0.48$ mg/ml, IC_{50} of total antioxidant capacity (TAC) evaluating by phosphomolybdenum assay of 26.323 mg/ml, 4-nitrophenylferrocene has the highest TAC estimated by the decrease of the current density of anodic peak of oxygen with 10.907 and the highest scavenging activity of superoxide radical with $IC_{50} = 1.16$ mg/ml.

Keywords: cyclic voltammetry, DPPH, superoxide anion, phosphomolybdenum assay

INTRODUCTION

The consumption of oxygen necessary for the growth and respiration of cells generates a series of reactive oxygen species (ROS), which include, the superoxide anion radical, peroxide anion, singlet oxygen and the hydroxyl radical [1], these reactive species which are responsible for cell damage are the cause of the aging process and the progression of the disease such as cancer, cardiovascular disease, rheumatoid arthritis, various respiratory diseases, eye diseases, Alzheimer's disease [2].

The Human Body protection system which functions to neutralize free radicals involves: enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase, they catalyze the reactions of inhibition of free radicals, antioxidants derived nutrients such as ascorbic acid (vitamin C) and tocopherols (vitamin E) and other molecules such as ferritin, albumin and lactoferritin [3].

Researchers do not cease to develop new synthetic molecules that exhibit antioxidant activity to be used in several industries like the food and cosmetics industry.

Ferrocene, discovered in 1951 by Pauson and Kealy [4], is considered the first product that launched the organometallic chemistry, and has since remained a molecule of interest because of its chemical properties.

Research has shown that the ferrocene derivatives has a variety of applications in various fields and serve as cytotoxic agents [5, 6], anti tumor [7, 8], antifungal [9], anti-malarial [10] and anti-oxidants [11-14].

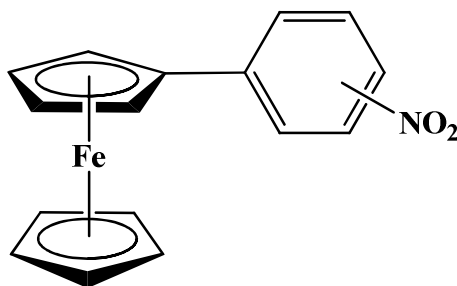
In this work, antioxidant capacities of two ferrocene derivatives; 2-nitrophenylferrocene, 4-nitrophenylferrocene were investigated, the antioxidant activity of these compounds was evaluated by electrochemical (the decrease in the current density of the anodic peak of superoxide anion using cyclic voltammetry), and chemical assays (phosphomolybdenum assay, DPPH assay).

EXPERIMENTAL SECTION

Chemicals : Methanol (99%), Potassium chloride (99.8%), Dimethylformamide (99.9%) were purchased from Biochem Chemopharma Co(Canada), Dimeyhulsulfoxide (99.9%), Tetrabutylammonium tetrafluoroborate (99%) from Sigma-Aldrich Co(Switzerland), α -tocopherol (97%), 1,1-Diphenyl-2-picryl hydrazyl (DPPH) (99%) were all purchased from Merck Co.

Instrument: UV-Visible spectrophotometer (PRIM Advanced SCHOTT instruments GmbH), PGZ301 potentiostat with Voltmaster 4 version 7.08 software (radiometer analytical SAS).

Synthesis: 2-nitrophenylferrocene (2-NPF) and 4-nitrophenylferrocene (4-NPF) were prepared by the diazonium salt method [15], scheme 1.



Scheme 1. Synthesis of N nitrophenylferrocene 2-NPF: X = 2-NO₂, 4-NPF: X = 4-NO₂

Determination of antioxidant activities:

DPPH radical scavenging activity:

The inhibition of DPPH was assessed by scavenging of 2,2-diphenyl-1-picrylhydrazyl radical according to the method of Brand-williams with slight modification [16]. A solution of DPPH was made by dissolving 0.985 mg of DPPH in 25 ml of methanol. The DPPH solution was diluted with methanol to obtain an absorbance of around 1.00 at 517 nm. 0.1 ml of a certain concentration of ferrocene compounds dissolved in dimethylsulfoxide (DMSO) was added to 1.9 ml of DPPH solution [17]. Absorbance was recorded at 517 nm after 30 min of incubation in the dark. The same experiment was carried out on the standard α -tocopherol. All test and analysis were run in triplicates to check their reproducibility of the obtained results. The DPPH scavenging activity of various compounds was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_c is the absorbance of control without sample and A_s is the absorbance of sample.

Free superoxide anion scavenging activity

The effect of scavenging superoxide radical was determined by cyclic voltammetry based on the method of Le Bourvellec et al. with slight modification [18].

Cyclic voltammetric measurements were performed using a Voltalab 40 model PGZ301 (Radiometer Analytical) potentiostat/galvanostat driven by a personal computer with VoltaMaster 4 software. The electrochemical cell (V=25 ml) consists of three electrodes immersed in a solution containing the analyte and the supporting electrolyte (Bu₄NBF₄). A saturated calomel electrode (SCE) was used as the reference electrode, a platinum wire as the auxiliary electrode, and a glassy carbon electrode ($\varnothing=3.0$ mm) as the working electrode.

Prior to use, the working electrode was polished, rinsed with distilled water, and dried with the absorbent paper. The superoxide anion radical was generated by one electron reduction of commercial molecular oxygen dissolved in DMF containing Bu₄NBF₄ at room temperature (28±1°C). The scan rate was maintained at 100 mV/s. The applied potential range was from 0.0 to -1.6 V versus SCE. The studied ferrocene derivatives and the standard antioxidant α -tocopherol were added to the in situ generated superoxide anion radical and the cyclic voltammograms were recorded. The ability of the test sample to quench superoxide anion radicals (O₂⁻) was calculated using the following equation:

$$\% \text{ O}_2^- \text{ radical scavenging activity} = \frac{ip_0 - ip_s}{ip_0} \times 100 \quad (2)$$

Where ip_0 and ip_s are the anodic peak current densities of the superoxide anion radical in the absence and in the presence of test compounds.

Phosphomolybdenum assay

The antioxidant capacity was also evaluated by adapting the method described by Prieto et al. [19]. 100 μ l of each tested compound solution was mixed with 1.0 ml of the reagent solution, consisting of phosphate buffer, 0.6 M H_2SO_4 , 28 mM sodium molybdate and 4 mM ammonium molybdate. The mixture was incubated in a water bath at 95°C for 90 min. After cooling to room temperature, the absorbance was taken at 695 nm. Total antioxidant capacity was expressed as equivalents of α -tocopherol using the following linear equation:

$y = 2.2533x + 0.0977$ where y is the absorbance at 695nm and x the concentration of α -tocopherol equivalent mg/ml [20].

Decrease in oxidation peak current of oxygen

The total antioxidant activity of the two studied ferrocene derivatives was estimated by the method of T. Lanez and A. Rebiai [21]. The decreasing in the anodic peak current density on the cyclic voltammograms of oxygen in the absence and in the presence of the ferrocene derivatives was measured; the total antioxidant capacity was then calculated using the following equation:

$$\text{Total antioxidant capacity} = \frac{ip_0 - ip}{ip_0 - ipres} \times 100 \quad (3)$$

($ip_0 - ip$) is the change in the anodic peak current density of oxygen caused by the addition of the sample, ($ip_0 - ipres$) is the difference between the limiting anodic peak current density of oxygen without the sample and the residual current density of the oxygen.

Statistical analysis

The results were presented as the mean values \pm SD. Correlation analyses between the antioxidant activities were carried out using the correlation and regression program in the EXCEL program.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The reducing capacity of an antioxidant was determined by their interaction with the free stable radical 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) at different concentrations. Antioxidants can react with DPPH and produce 1, 1-diphenyl-2-picryl-hydrazine as in figure (1) [22].

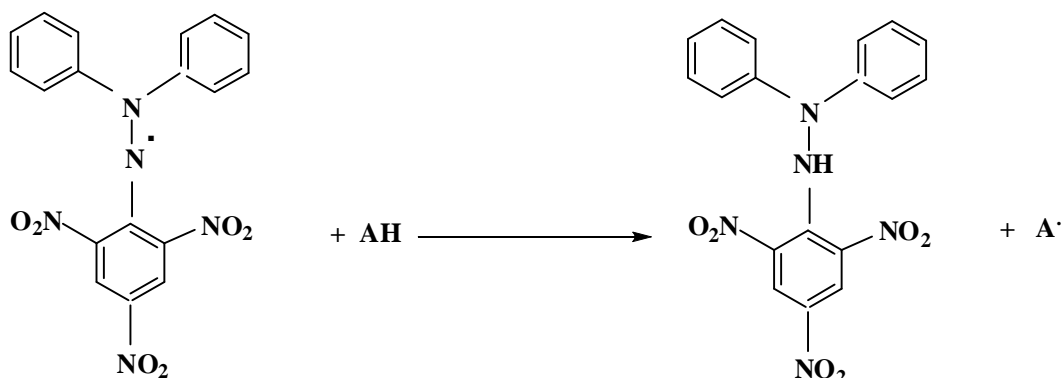


Figure 1. Reaction of DPPH· free radical with an antioxidant AH

The antioxidant activities obtained by the DPPH method for the ferrocene derivatives are displayed in Table 1. The studied compounds showed scavenging activity and the best results were obtained with 2-NPF (0.48 mg/ml). The DPPH radical scavenging activities (IC_{50}) of the tested compounds were in order: 2-NPF < 4-NPF.

Superoxide anion radical

The superoxide anion radical was generated by one electron reduction of the commercial molecular oxygen (O_2) dissolved in DMF at room temperature ($28^\circ C \pm 1$) and the resultant CV response.

The reduction of O_2 is a reversible reaction and it is known that $O_2^{\cdot-}$ radical is stable in aprotic media and dismutation does not occur during the time scale of the cyclic voltammetry in DMF solution [19].

The obtained results (figure 2) show that in the two cases, the addition of the studied compound causes a proportional decrease of $O_2^{\cdot-}$ anodic peak current while the intensity of O_2 cathodic current appears to be negligible. Figure (3) shows the cyclic voltammograms of α -tocophérol in the same conditions.

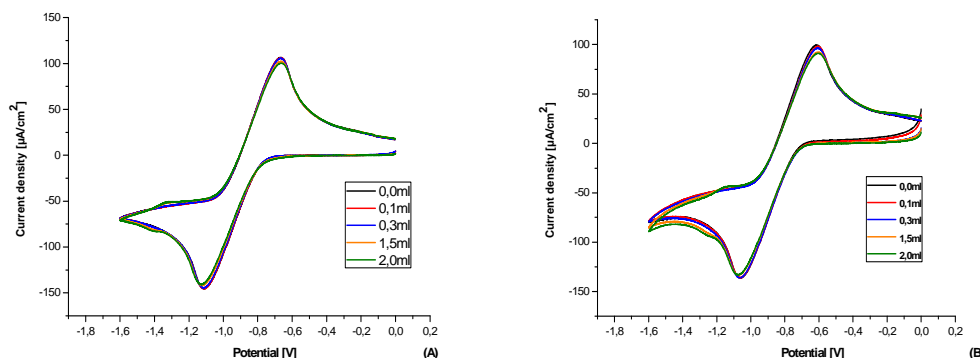


Figure 2. Cyclic voltammograms of $O_2^{\cdot-}$ in the presence of different concentration of 2-NPF (A) and 4-NPF (B) in DMF + 0.02 M Bu4NBF4 on GC as working electrode vs. SCE at $28 \pm 1^\circ C$ with scan rate of 0.1 V/s

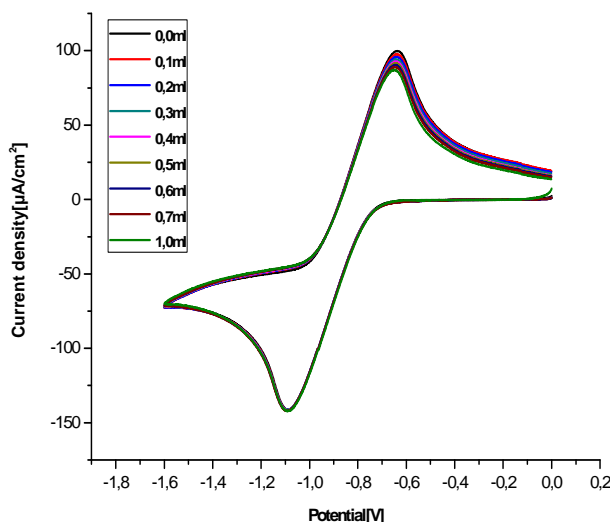


Figure 3. Cyclic voltammograms of $O_2^{\cdot-}$ in the presence of different concentration of α -tocophérol in DMF + 0.02 M Bu4NBF4 on GC as working electrode vs. SCE at $28^\circ C$ with scan rate of 0.1 V/s

The decrease of the anodic peak current of $O_2^{\cdot-}$ suggests that the ferrocene derivative reacts irreversibly with $O_2^{\cdot-}$. For each antioxidant compound, a series of values is determined from the CVs recorded for increasing antioxidant concentrations (Table1).

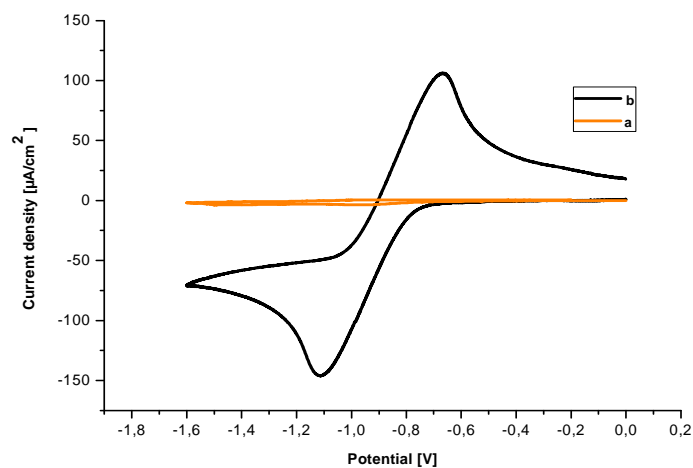
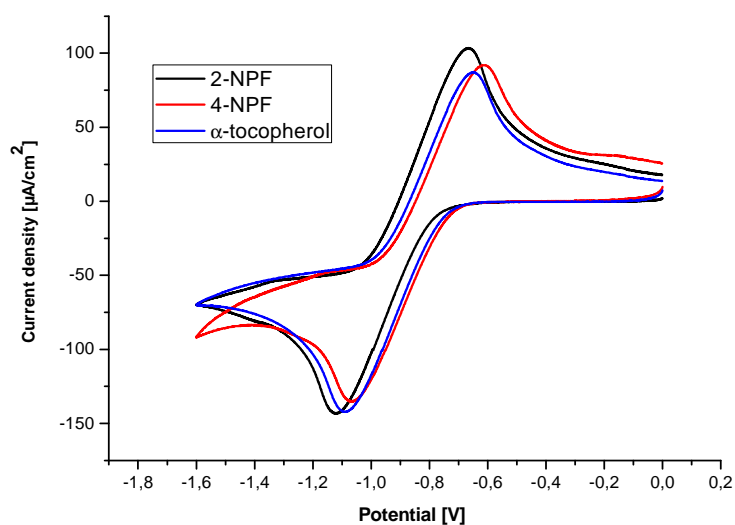
The scavenging activity of the antioxidant was evaluated by its IC_{50} , it is defined by the concentration which inhibits 50% of the radical and calculated from the linear equation (y: the percentage antioxidant activity and x: compound concentration (Fig 04). From the table 1, It can be seen that the IC_{50} values of ferrocene derivatives showed an antioxidant capacity. The lowest value of IC_{50} (0.48 mg/ml) was detected in 2-NPF and it corresponds to the highest antioxidant activity; while the highest value of IC_{50} (1.57 mg/ml) was detected in 4-NPF.

Table 1. IC₅₀ values of the ferrocene derivatives and α -tocopherol obtained by the DPPH and superoxide anion inhibition tests

Compound	Methods	Equation	R ²	IC ₅₀ (mg/ml)
2-NPF	DPPH	$y = 92.64x + 4.844$	0,951	0,48
	O ₂ .-	$y = 20.45x + 0.319$	0,994	2,43
4-NPF	DPPH	$y = 30.77x + 1.414$	0,988	1,57
	O ₂ .-	$y = 41.36x + 1.986$	0,942	1,16
α -tocophero	DPPH	$y = 1.56x + 29.093$	0,995	0,0134
	O ₂ .-	$y = 2.192x + 1.692$	0,939	0,016

Phosphomolybdenum assay

The phosphomolybdenum assay was employed to estimate the total antioxidant capacity of the different compound. The phosphomolybdenum method was based on the principle of the reduction of Mo (VI) to Mo (V) by the different antioxidants and the formation of green phosphate/Mo (V) complex [20]. Table (2) shows that the highest antioxidant capacity was exhibited by the 2-NPF. It having IC₅₀ of TAC value of 26.323 VEE mg/ml followed by 4-NPF with IC₅₀ value of 134.33VEE mg/ml.

Figure 4. Cyclic voltammogram of: (a) medium (DMF + Bu₄NBF₄), (b) O₂⁻ in DMF + 0.02 M Bu₄NBF₄, on the glassy carbon as working electrode vs. ECS as reference with scan rate of 0.1Vs⁻¹Figure 5. Cyclic voltammograms of O₂⁻ in the presence of 1.0 ml of 2-NPF, 4-NPF and α -tocopherol, in DMF + 0.02 M Bu₄NBF₄ on GC as working electrode vs. SCE with scan rate of 0.1 V/s

Decrease in anodic peak current of oxygen:

The almost methods of evaluating antioxidant capacity described in literature are based on the use of IC₅₀. As advantage, this simple method evaluate the total antioxidant capacity (TAC) without need to calibration curve or standard antioxidant using a cyclic voltammery technique, it based on the measure of the change in anodic peak current and the residual current density of superoxide anion after injection of 1.0 ml of the studied sample.

The cyclic voltammograms of the superoxide anion radical in the presence of tested ferrocenic compounds: 2-NPF and 4-NPF (figure 5) showed a decrease in the anodic current, this decrease in the anodic current of superoxide anion radical is attributed to the presence of antioxidant which react with this radical and decrease its concentration around the electrode surface. Antioxidant activity of the studied compounds is calculated with the equation (03).

Table 2. Total antioxidant capacities of the ferrocene derivatives and α -tocopherol obtained from molybdate ion reduction and decrease in anodic peak current of superoxide anion

Compound	$I_{p_0} - I_p$ ($\mu\text{A}/\text{cm}^2$)	$I_{p_0} - I_{p_{res}}$ ($\mu\text{A}/\text{cm}^2$)	IC ₅₀ of TAC (VEE mg/ml)	TAC (O ₂ ⁻) (%)
2-NPF	2,3	105,07	26.323 \pm 0.087	2.178 \pm 1.0040
4-NPF	11,46	105,07	134.33 \pm 0.256	10.907 \pm 1.74979
α -tocopherol	18,38	105,07	/	17.493 \pm 0.50595

CONCLUSION

Two ferrocene derivatives were successfully synthesized and their antioxidant activity was studied using spectrophotometrical and electrochemical assays. Both assays reviled a measured antioxidant activity for both compounds. The obtained results indicate that the scavenging activity of the two studied compounds gradually increased in a concentration-dependent manner.

2- nitrophenylferrocene exhibit a higher antioxidant activity even higher than antioxidant standard α -tocopherol.

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