# Voltammetric redox behavior of nitrofuryl 1,4-dihydropyridine derivatives: Interdependence between two redox centers

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**Abstract**

To investigate new chemotherapeutics alternatives to Chagas' disease, three 4-(5′-nitro-2′-furyl)-1,4-dihydropyridine derivatives were synthesized and electrochemically investigated in order to clarify their redox behavior. The redox behavior of these compounds is very attractive as they share a reducible (nitrofuryl) and an oxidizable (1,4-DHP) moiety, the interdependence of these two redox centers will be our current challenge. The reduction of the nitrofuryl derivatives was studied in two different protogenic aqueous-organic media (Britton–Robinson buffer/ethanol: 70/30 and citrate buffer/DMF: 60/40) and working at two different experimental time windows (i.e., Polarographic and cyclic voltammetric experiments). The current work reveals that both redox centers showed a great interdependence and electronic communication, determining its electrochemical properties. The reduction potential of the nitrofuryl moiety can be modulated at both fine tuning (tens of mV) by changing the electronic properties of the 3,5 substituents in the 1,4-DHP ring, and coarse tuning (cents of mV) by changing the oxidation state of the 1,4-DHP ring into a pyridine ring, thus producing nitrofuryl derivatives with very low reduction potentials, i.e., good candidates to be reduced by nitroreductases.

*Keywords:* Two redox centers; Nitrofuryl; Electro-reduction; 1,4-Dihydropyridine

## 1. Introduction

Chagas' disease continues to be a serious threat to health in many Latin American countries [1]. Nifurtimox and benzanidazole are nitroheterocyclic drugs currently used to treat this sickness; however, they have generated resistance [2]. Then, the chemotherapy of Chagas' disease is still an open field and remains as an unsolved problem [3]. Therefore, we have synthesized a new series of nitrofuryl substituted 1,4-dihydropyridine derivatives [4,5]. The mechanism of most nitroheterocyclic drugs is associated to metabolic reduction of their nitro group [6]. Specifically, the nitro radical anion formation seems to be a determining step and consequently the knowledge of its chemistry is crucial for its application in medicine [7]. Electrochemistry plays an important role to study the formation of this radical and its reactivity in one-pot systems [8].

Although the electro-reduction behavior of nitroaromatic compounds has been extensively studied; the electro-reduction of nitrofuryl derivatives has been a rather scarcely touched topic [9]. In fact, the reduction potentials of nitrofurans were examined in the late 1960s using DC polarography [10]. Later, in the 1990s other works on the cyclic voltammetric behavior of some nitrofurans derivatives such as nitrofurazone, nifuroxime, nitrofurantoin and furazolidone [11–13] were published. More recently the nitro radical anion formation from nifurtimox and nifuroxazide [14,15] and the interaction of the nitro radical anion from nitrofurazone and related compounds have been voltammetrically approached [16,17]. Furthermore, the nitro radical anion from nitrofuryl compounds has been detected in different electrode materials such as Hg [17], glassy carbon [18] and boron-doped diamond electrodes [19].

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The electrochemistry of nitrofuryl 1,4-dihydropyridine derivatives has become an attractive subject as they share groups with reducible and oxidizable character within the same molecule. In a first study, we reported the oxidation of these molecules in an aqueous medium demonstrating the formation of the pyridine derivative in a strongly pH-dependent mechanism [4]. A second study focused on its electro-reduction in non-aqueous medium, revealing the generation of a nitranion species as a consequence of the father–son type reaction between the nitro radical anion and the parent compound [5].

In the present study, we have focused on the electro-reduction of 4-(5′-nitro-2′-furyl)-1,4-dihydropyridine derivatives in two different aqueous-organic media (Britton–Robinson buffer/ethanol: 70/30 and citrate buffer/DMF: 60/40) and working at two different experimental time windows in order to study their influence on the visualization of the nitro radical anion. Furthermore, considering the dual redox character of the studied molecules we have also investigated the interdependence of both redox centers in their electrochemical character.

## 2. Experimental

The synthesis of the nitrofuryl 1,4-dihydropyridine derivatives (Fig. 1) has been previously described [4]. Stock solutions of 0.01 M were prepared in ethanol or DMF. The working solutions were prepared by diluting the stock solution until final concentrations of 0.5 and 1 mM. Voltammetric and polarographic experiments were carried out in two different media: (a) Britton–Robinson buffer with 30% ethanol as co-solvent and 0.3 M KCl as supporting electrolyte and (b) citrate buffer with 60% DMF and 0.3 M KCl plus 0.1 M tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. Ultrapure water (Milli-Q) was employed in all experiments. All used reagents were of analytical grade. The solutions were purged with nitrogen for at least 10 min before each measurement.

Differential pulse (DPP), Tast (TastP) and cyclic voltammetric (CV) measurements were performed at $25 \pm 1$ °C with a BAS-CV 50 electrochemical analyzer. The reference electrode, was Ag/AgCl, KCl(Sat.), and the counter electrode was a Pt wire. A mercury drop electrode (controlling growth mercury electrode, CGME stand of BAS) was used as working electrode. For DPP and TastP the CGME stand was used in a CGME mode and for CV experiments the CGME stand was used as SMDE mode (static mercury drop electrode), whereas a mercury pool electrode was employed for the coulometric analysis. For voltammetric measurements in the anodic range a glassy carbon electrode (GCE) as the working electrode was used.

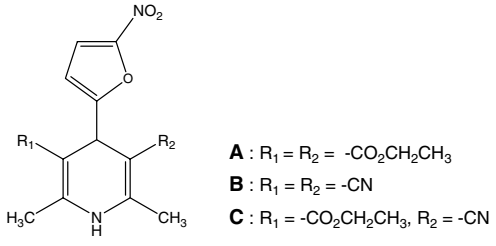



Fig. 1. Chemical structure of 4-(5′-nitro-2′-furyl)-1,4-dihydropyridine derivatives.

pH measurements were done with a WTW microprocessor controlled standard-pH-ion meter pMX 3000/pH equipped with a glass pH-electrode SenTix 81.

pH Measurements were corrected according to a procedure reported previously [20,21].

## 3. Results and discussion

In aqueous Britton–Robinson buffer/ethanol: 70/30, the pH influence on the polarographic response was recorded between pH 2 and pH 12. Fig. 2A and B shows that DPP and TastP curves are strongly affected by pH, moving to more negative potentials by pH increase. Fig. 2C reveals that at pH < 9 the reduction process is pH-dependent; and pH-independent above this pH value. At alkaline pH, both TastP and DPP curves become very wide and badly resolved resembling two overlapped signals. Fig. 2D shows that the limiting current obtained by TastP is relatively constant in all pH values, indicating that equal numbers of electrons were transferred. Furthermore, according to coulometric analysis, four-electrons are consumed in the reduction process at pH 7 (Table 1).

Consequently, the compounds showed a four-electron signal corresponding to the reduction of the nitro group to the hydroxylamine derivative according to:

$$\text{R-NO}_2 + 4e^- + 4H^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O} \quad (1)$$

At pH < 5, only compound B showed a second reduction wave at more negative potentials (−0.35 to −0.45 V) attributed to the reduction of the protonated hydroxylamine to amine derivatives according to:

$$\text{R-NH}_2\text{OH}^+ + 2e^- + H^+ \rightarrow \text{R-NH}_2 + \text{H}_2\text{O} \quad (2)$$

The above behavior is similar to that previously described for nitrobenzene [9] confirming an aromatic character for this nitrofuryl moiety.

Table 1 also shows the direct relationship between the reduction potential (Ep) and the electron withdrawing effect of the substituent in 3,5 positions on the dihydropyridine ring. When the strongest electron acceptor groups are present, the nitro group was more easily reduced, showing a good correlation between the reduction potentials of the nitro group and the Hammett's substituent constants, $\sigma_I$ (0.34 for $-CO_2CH_2CH_3$ and 0.51 for –CN) on the dihydropyridine moiety. This finding reveals an electronic communication between both redox centers, which permits to modulate the potential to reduce the nitro group, an important parameter to have biological activity against *Trypanosoma cruzi*.

Comparatively, in the named pH-independent zone (pH > 9) we used a low protogenic medium (aqueous cit-

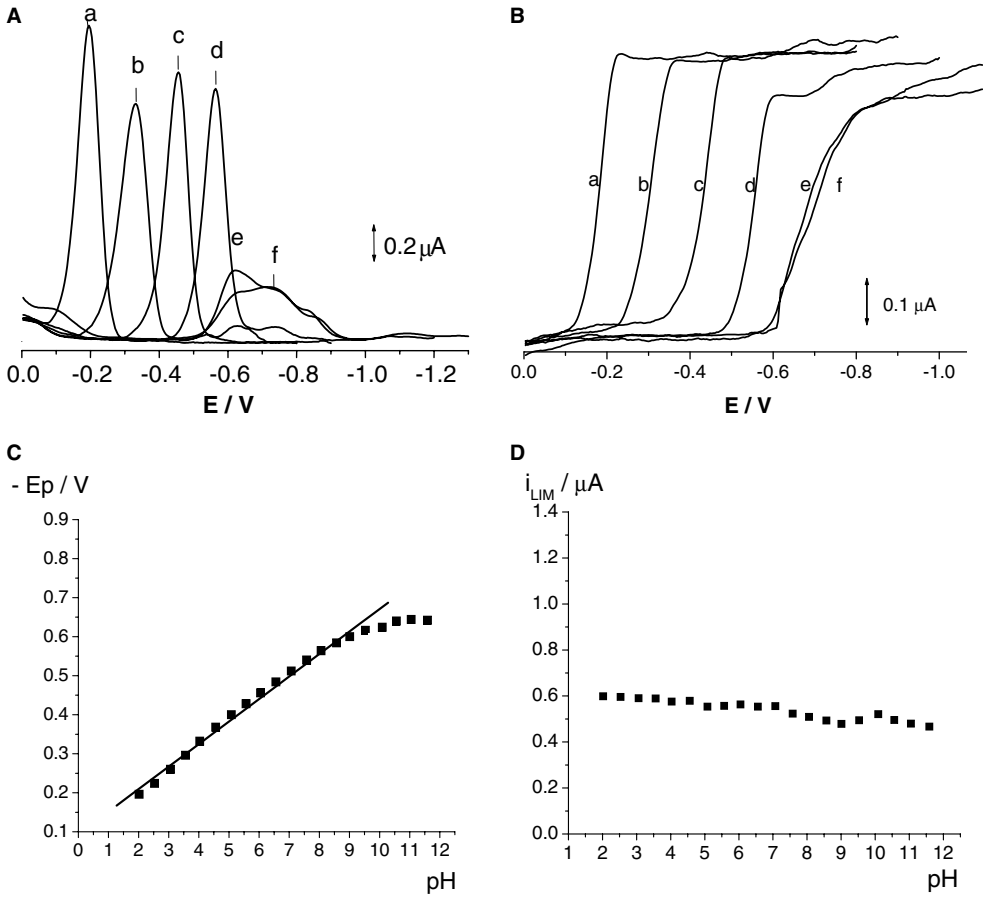



Fig. 2. (A) Differential pulse and (B) Tast polarograms of 0.5 mM of compound A in aqueous Britton–Robinson buffer/ethanol: 70/30, at different pH values: (a) 2, (b) 4, (c) 6, (d) 8, (e) 10 and (f) 11. pH-dependence of: (C) cathodic peak potential and (D) limiting current.

Table 1
Cathodic peak potentials obtained from DPP of nitrofuryl derivatives in aqueous Britton–Robinson buffer/ethanol: 70/30 at different pHs and coulometric $n$ values obtained at pH 7

| Compounds | $-E_{PC}$/V | | | $n$ |
|---|---|---|---|---|
| | pH 3 | pH 7 | pH 10 | pH 7 |
| A | 0.240 | 0.492 | 0.672 | $4.1 \pm 0.3$ |
| B | 0.160 | 0.392 | 0.540 | $4.1 \pm 0.3$ |
| C | 0.204 | 0.452 | 0.648 | $4.0 \pm 0.1$ |

rate buffer/DMF: 40/60) in order to obtain a better resolution of the signals (Fig. 3). Our hypothesis is that the ill resolution in that zone is due to the transition caused by a change in the redox behavior passing from four-electron reduction signal to a one-electron signal, as observed in aprotic medium [9]. Compared with the most protogenic medium, the peak potentials values shifted slightly toward more negative potentials, but following the same pH-dependent behavior in 2–9 pH range. The most significant difference between both media was observed at pH > 9, where the DPP curve displayed two well-separated reduction peaks (I, II) with $E_{PC}$ values remaining constant when pH increased (Fig. 3B). However, we used TastP to explain the observed behavior because it gives a proportional relationship between the electrons transferred and the height of the waves. Consequently, the change in the TastP waves in the 8–11 range of pH is showed in Fig. 3C. From this figure is possible to observe a 1:3 height ratio at strong alkaline pH proving the existence of a one-electron transfer to wave (I) and a three-electron transfer to wave (II). Furthermore, Fig. 3D shows the dependence of the TastP waves limiting currents on pH in all the pH range. From this plot is possible to observe the four-electron wave decrease (I) to a one-electron wave with increasing pH. Simultaneously, an irreversible three-electron reduction wave (II) arises at more negative potentials. From these experimental evidences is possible to conclude that in strong alkaline condition, wave (I) is attributed to the nitro radical anion formation and wave (II) to a subsequent three-electron reduction of the nitro radical anion to hydroxylamine derivative, according to:

$$\text{R-NO}_2 + e^- \rightleftharpoons \text{R-NO}_2^{\bullet -} \quad (3)$$

$$\text{R-NO}_2^{\bullet -} + 3e^- + 4H^+ \rightarrow \text{R-NHOH} + H_2O \quad (4)$$

In order to analyze the nitrofuryl derivative reduction but with another time window, CV was tried. In the higher protogenic medium (aqueous buffer/ethanol) CVs at pH 3 and pH 7 exhibited only one irreversible cathodic peak (I), due to nitro group reduction to hydroxylamine

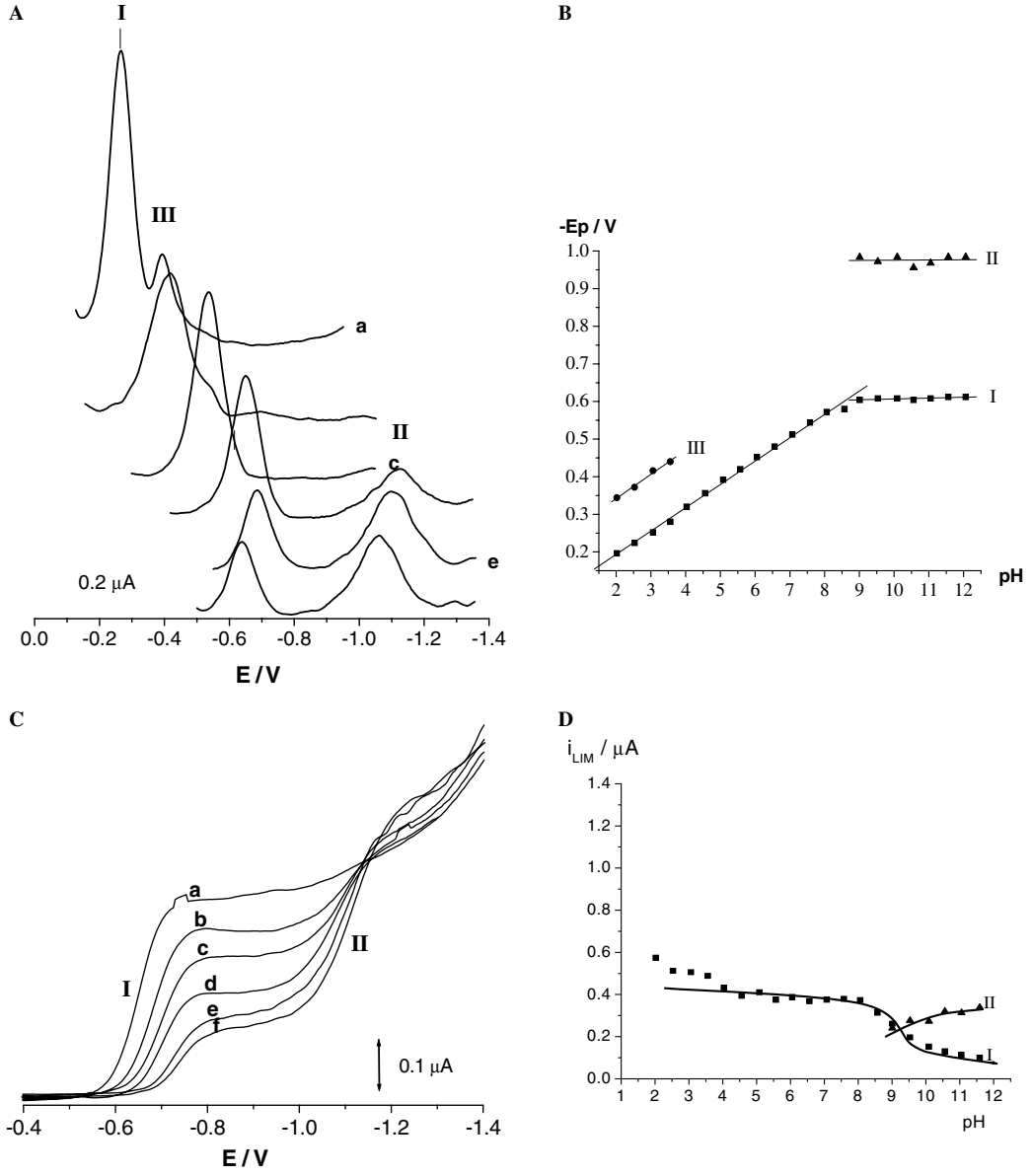



Fig. 3. (A) Differential pulse polarograms at different pH values: (a) 2, (b) 4, (c) 6, (d) 8, (e) 10 and (f) 12 and (B) the corresponding cathodic peak potential versus pH plot; (C) Tast polarograms at different pH values: (a) 8.0, (b) 8.5, (c) 9.0, (d) 9.5, (e) 10 and (f) 11 and (D) the corresponding limiting current versus pH plot. All the measurements were carried out on 0.5 mM of compound B, in aqueous citrate buffer/DMF: 40/60.

(Fig. 4A). The shoulders appearing at lower potentials at pH 3 and pH 7, correspond to the further reduction of the protonated hydroxylamine derivative to form the amine derivative (Eq. 2). However, at pH 9, two well-resolved peaks can be detected caused by electron transfer reactions described by Eqs. (3) and (4). When CVs at pH 9 (Fig. 4) are compared with the corresponding polarograms (Fig. 2), the separation of two well-resolved peaks was possible only for the CV curves. This could be explained by differences in sweep rates in both experiments, namely, the CV curves were obtained at least at 100 mV/s, while the polarographic curves, at 4 mV/s. This very different time window for both experiments produces a better resolution of the signal in CV experiments.

When we used the low protogenic medium at the same time window of the CV experiment, we improved the resolution for both signals. Fig. 4B, shows CV at pH 9, displaying one reversible couple (I/III) and one irreversible cathodic peak (II). The peak potential separation of the redox couple (I/III), ΔEp, for compounds A, B and C were 0.066, 0.062 and 0.061 V, respectively, which are close to the theoretical value of 0.060 V for kinetically reversible one-electron reaction, confirming the one-electron character of the first wave according to the above Eq. (3).

On the other hand, in order to deepen the interrelation between the redox properties of nitrofuryl and dihydropyridine moieties we used a glassy carbon electrode in order to first oxidize the 1,4-DHP and then reduce the nitro

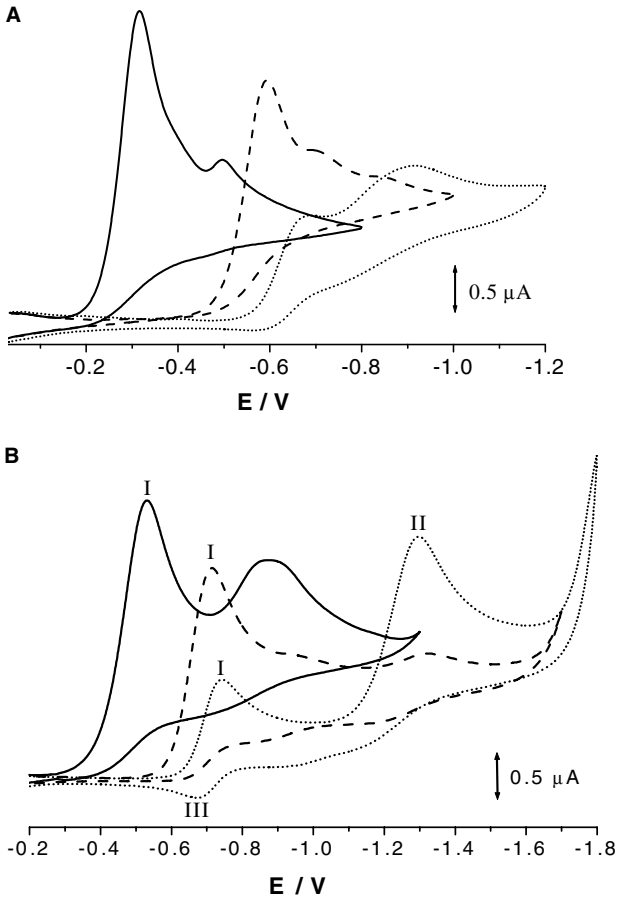



Fig. 4. Cyclic voltammograms of: (A) 1 mM of compound C in aqueous buffer-ethanol/70:30, at pH: 3 (solid line), pH 7 (dashed line) and pH 9 (doted line). Scan rate: 0.1 V $s^{-1}$ and (B) 1 mM of compound A, in aqueous citrate buffer/DMF: 40/60 at pH 3 (solid line), pH 7 (dashed line) and pH 9 (dotted line). Scan rate: 0.1 V $s^{-1}$.

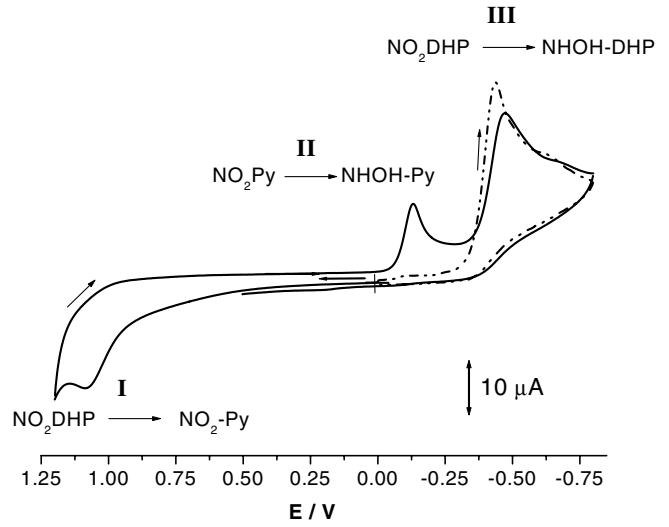
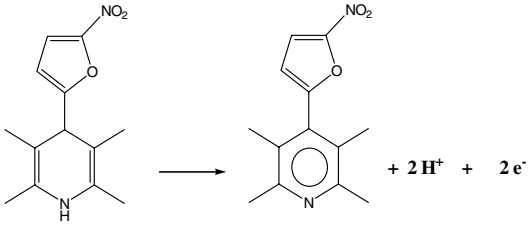


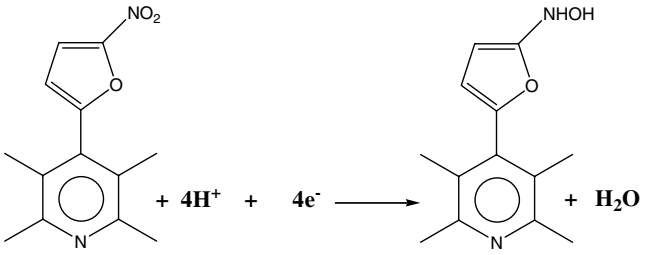

Fig. 5. Cyclic voltammograms on glassy carbon electrode of 1 mM of compound B, in aqueous citrate buffer/DMF: 40/60 at pH 3. Only a cathodic sweep starting at 0.0 V (doted line); anodic–cathodic sweep, initial potential: 0.0 V, switching potential: 0.12 V, final potential: −0.80 V. Scan rate: 0.1 V $s^{-1}$.

group in the same run. Fig. 5 shows the voltammograms of compound B in aqueous citrate buffer/DMF: 40/60 starting at 0.0 V with an anodic sweep and then reversing the sweep at 1.20 V in order to carry out a further cathodic sweep. From the voltammogram we can identify a first anodic peak, peak I, due to the oxidation of the dihydropyridine moiety to pyridine according to the following equation:




(5)

The oxidation of compounds A, B and C leading to the formation of a pyridine moiety has been previously demonstrated by electrolysis followed by GC-MS [4]. In the cathodic sweep we observe two peaks, wherein peak III corresponds to the reduction of the nitrofuryl moiety in the nitrofuryl 1,4-dihydropyridine derivative, and the cathodic peak II would correspond to the reduction of the nitrofuryl in the previously generated nitrofuryl pyridine derivative according to the following equation:




(6)

A comparison of the nitrofuryl reduction potentials between the dihydropyridine and pyridine derivatives permits to detect a difference in about 0.35 V, showing that the aromatization of the dihydropyridine moiety facilitates the electro-reduction of the nitro group. This difference can be attributed to a change in the C-4 hybridization in the DHP ring from $sp^2$, which is more electronegative, to $sp^3$. This could be a quite important characteristic because the compounds could be capable to be reactive to both oxidases and reductases enzymes. Consequently, this type of compounds could act as antichagasic pro-drugs, where in a first step the compound would be oxidized by the corresponding enzymes in the host facilitating a further reduction at considerable lower energy requirements in the parasite. The presence of 1,4-DHP moiety instead of a pyridine moiety in a possible drug is highly preferred because of the proved high efficiency as carriers for specific drug delivery [22].

## 4. Conclusions

Although the nitrofuryl moiety is not a typically aromatic group, according to the above results, we concluded

that from the electrochemical point of view its behavior was that of a nitroaromatic group. Whereas in both studied media at pH < 9 reduction occurs in a single four-electron step, at pH > 9 the situation varied depending on proton activity of the media and the time window of the experiments. The visualization of the nitro radical anion signal was improved both by low protogenic activity of the medium and by shortening the time window of the experiments.

According to previous [4,5] and the above results, it is possible to conclude that the studied nitrofuryl substituted 1,4-DHP has a dual redox behavior due to the electro-reductive nitrofuryl moiety and the electro-oxidizable dihydropyridine group. Furthermore, both redox centers showed a great redox interdependence and electronic communication, determining the electrochemical properties of these compounds. Hence, the easiness of reduction on the nitrofuryl moiety can be modulated by either changing the electronic activity of the 3,5 substituent on the 1,4-DHP ring or by previous oxidation of 1,4-DHP.

These results may be an electrochemical support for a new concept of antichagasic pro-drugs that would act in a two-step mechanism with a first step involving the oxidation of the 1,4-DHP to the pyridine moiety and then the further reduction of the nitrofuryl moiety of the already oxidized molecule at considerable lower reduction potential. Evidently, a compound with a pyridine moiety as the initial component of the molecule would produce the same low reduction potential on nitrofuryl moiety, but the presence of 1,4-DHP moiety instead of pyridine moiety in the molecule would permit better in vivo absorption and distribution of the molecule, providing a compound with enhanced pharmacokinetic characteristics.

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