Nitro radical anion formation from nitrofuryl substituted 1,4-dihydropyridine derivatives in mixed and non-aqueous media

J. Argüello, L.J. Núñez-Vergara, S. Bollo, J.A. Squella *

Bioelectrochemistry Laboratory, Chemical and Pharmaceutical Sciences Faculty, University of Chile, POB 233, Santiago 1, Chile

Abstract

Three new nitrofuryl substituted 1,4-dihydropyridine derivatives were electrochemically tested in the scope of newly found compounds useful as chemotherapeutic alternative to the Chagas' disease.

All the compounds were capable to produce nitro radical anions sufficiently stabilized in the time window of the cyclic voltammetric experiment. In order to quantify the stability of the nitro radical anion we have calculated the decay constant, k_2. Furthermore, from the voltammetric results, some parameters of biological significance as E_7^1 (indicative of in vivo nitro radical anion formation) and K_{O2} (thermodynamic indicator of oxygen redox cycling) have been calculated. From the comparison of E_7^1, K_{O2} and k_2 values between the studied nitrofuryl 1,4-DHP derivatives and well-known current drugs an auspicious activity for one of the studied compounds i.e. FDHP2, can be expected.

Keywords: Nitro radical anion; Nitrofuryl, 1,4-dihydropyridine; Chagas' disease; Decay constants

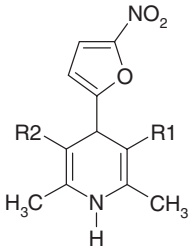
1. Introduction

Trypanosoma cruzi is the etiological agent of Chagas' disease, a chronic illness affecting many people in Latin America [1]. Currently, this pathology is treated with nitroheterocyclic agents such as nifurtimox (nitrofuran derivative) and benznidazole (nitroimidazole derivative) which act against the circulating form of the parasite (trypomastigotes) during the acute phase of the disease. Several evidences [2,3] suggest that intracellular reduction produces the nitro radical anion derivative which acts directly or indirectly generating superoxide anion to eliminate the parasite. Regrettably these drugs produce serious adverse effects including mutagenesis [4,5], after use of long term therapy. Consequently, the chemotherapy of Chagas' disease is still an open field wherein the replacement of the current drugs with new ones with fewer adverse effects is an urgent challenge. Progress towards the development of novel therapeutics can be obtained through of a rational drug design.

In the scope of our investigations tending to find new therapeutic alternatives to the treatment of Chagas, we have studied several nitroimidazole derivatives such as Megazol and related compounds [6,7]. However, it has been recently demonstrated that, megazol [8] is a potent inducer of in vitro and in vivo chromosomal aberrations. Although Megazol is a potent trypanocidal agent and is orally bioavailable, its toxicity dictates that it should not be used further for the treatment of Chagas' disease. Consequently we have focused our attention to a completely new type of compounds which share a nitrofuryl and a 1,4-dihydropyridine moiety in its structure [9]. Our hypothesis on this type of compounds is that the nitrofuryl moiety will be capable to generate the nitro radical anion, an intermediate able to attack the parasite, whereas the 1,4-DHP moiety would provide adequate bioavailability. The synthesis of some new nitrofuryl derivatives with activity as anti-*Trypanosoma cruzi* has been recently informed showing the renewal interest for the synthesis of this type of compounds [10,11].

The electrochemistry of nitrofuryl derivatives has been reported since middle of the preceding century. The reduction potentials of nitrofuryl derivatives were examined in the late 1960's using DC polarography [12]. Several other works related

* Corresponding author. Tel.: +56 2 9782927; fax: +56 2 7371241.
E-mail address: asquella@ciq.uchile.cl (J.A. Squella).



Compounds	R_1	R_2
FDHP1	- CO_2Et	- CO_2Et
FDHP2	- CN	- CN
FDHP3	- CO_2Et	- CN

Fig. 1. Molecular structure of nitrofuryl 1,4-dihydropyridine derivatives.

to the cyclic voltammetric behavior of nitrofuryl derivatives have been published [13–15]. More recently a cyclic voltammetric study on the nitro radical anion generated from some nitrofuryl derivatives of pharmacological significance has been reported [16]. Such article illustrates the powerful role that cyclic voltammetry can play in the better understanding of generation, stability and reactivity of nitro radical anion.

Recently, we have synthesized and studied the electroreduction of a new type of nitrofuryl derivatives substituted with a 1,4-dihydropyridine (1,4-DHP) moiety [9]. The one-electron reduction of these compounds in non-aqueous medium generated an stable nitro radical anion, however, the existence of an acidic proton on the 1,4-DHP ring triggered the appearance of father–son type reactions between the nitro radical anion and the parent compound, generating a nitranion species on the 1,4-DHP ring. In that paper we centered our attention in the formation reaction of the nitranion, but now we are interested in both the study of the nitro radical anion and how the nitranion can affect the stability of the nitro radical anion.

Considering that both, pharmacological and toxic effects, are mediated by cytotoxic reactions and depend on the in vivo reduction of the nitro group producing the nitro radical anion species ($R–NO_2^{\bullet -}$), in the present paper we will study the $R–NO_2^{\bullet -}$ formation and its stability from three nitrofuryl substituted 1,4-DHP derivatives in mixed and aprotic media. Thus, the stability constants corresponding to the nitro radical anion species in both media and the incidence of the nitranion on the stability of such species will be studied.

2. Experimental

2.1. Reagents and solutions

Ethyl 4-(5′-nitro-2′-furyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (FDHP1), 3,5-dicyano-4-(5′-nitro-2′-furyl)-2,6-dimethyl-1,4-dihydropyridine (FDHP2) and ethyl 3-cyano-4-(5′-nitro-2′-furyl)- 2,6-dimethyl 1,4-dihydropyridine-5-carboxylate (FDHP3) (Fig. 1) were synthesized in our laboratory according to the general procedure previously informed. The synthesized compounds were characterized by ^1H NMR, ^{13}C NMR spectroscopy using a 300 MHz spectrometer (Bruker, WM 300), infrared spectroscopy (FT-IR Paragon Spectrometer, 100PC) and Elemental Analysis (Perkin Elmer, 240 B) [9].

DMF was dried with 3 Å molecular sieves. All the other reagents employed were of analytical grade.

Stock solutions of each compound were prepared at a constant concentration of 0.025 M in DMF. The polarographic and cyclic voltammetric working solutions were prepared by diluting the stock solution until final concentrations of 0.5 and 1 mM, respectively, were obtained. Dimethylformamide (DMF) and 0.1 M tetrabutyl ammonium perchlorate (TBAP) as solvent and supporting electrolyte for aprotic medium and a mixture of 60/40:DMF/citrate buffer (KCl 0.3 M) for a mixed medium were used as reaction media.

2.2. Apparatus

Electrochemical experiments were performed with a totally automated BAS CV-50W voltammetric analyzer. All experiments were carried out at a constant temperature of 25 ± 0.1 °C using a 10 ml thermostated cell. A mercury drop electrode (controlling growth mercury electrode, CGME stand of BAS) with a drop area of 0.42 mm^2 as working electrode and a Pt wire as a counter electrode were used. All potentials were measured against Ag/AgCl (Sat).

For differential pulse (DP) and tast polarography, the CGME stand was used in a CGME mode and for cyclic voltammetric (CV) experiments, the CGME stand was used as SMDE mode (static mercury drop electrode).

Simulated CV curves were obtained using the software DIGISIM ® 2.1 CV simulator for Windows. Software was run using a Gateway 2000 PC.

All pH measurements were carried out with a WTW microprocessor controlled standard-PH-ion meter pMX 3000/

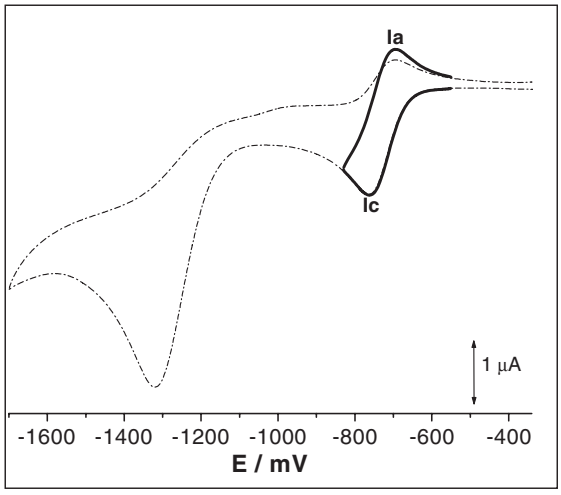


Fig. 2. Cyclic voltammogram of 1 mM FDHP1, in mixed medium at pH 9. Sweep rate 1 V s^{-1}. Whole line shows a short sweep with the isolated $RNO_2/RNO_2^{\bullet}$ couple.

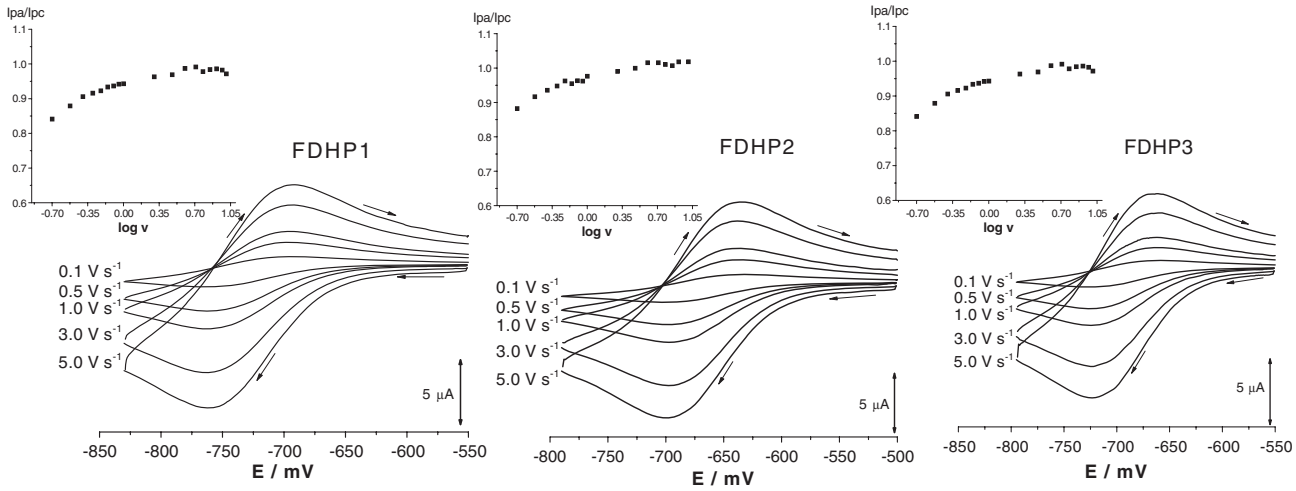


Fig. 3. Cyclic voltammograms of the isolated $RNO_2/R–NO_2^{\bullet-}$ couple from 1 mM of: a) FDHP1, b) FDPH2 and c) FDPH3 in mixed medium, 60/40: DMF/citrate buffer (KCl 0.3 M), at different sweep rates. Inset: dependence of the current ratio on sweep rate for each set of experiments.

pH equipped with a glass pH-electrode SenTix 81. The standard solutions used for calibration were WTW 4.006, 6.865 and 9.180.

2.3. Methods

For the kinetic analysis carried out in alkaline pH, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible one electron couple ($RNO_2/R–NO_2^{\bullet-}$) was measured for each cyclic voltammogram according to the procedure described by Nicholson [17]. The scan rate was varied between 0.1 and 10 V s^{-1}.

Using the theoretical approaches of Olmstead et al. for dimerization or disproportionation [18,19], the I_{pa}/I_{pc} values measured experimentally at each scan rate were inserted into a working curve to determine the parameter ω, which incorporates the effects of the rate constant, nitro compound concentration and scan rate.

3. Results and discussion

In a previous paper [9] we visualized the formation of nitro radical anions from the electroreduction in non-aqueous medium of all the nitrofuryl substituted 1,4-dihydropyridine derivatives (FDHPs) but in that study we focused the attention to the father–son type reaction between the nitro radical anion ($R–NO_2^{\bullet-}$) and the respective parent compound (RNO_2). Consequently a deep study of the nitro radical anion formation and its stability is a pending challenge.

3.1. Mixed medium

Fig. 2 shows the CV of FDHP1 in mixed medium (60/40: DMF/citrate buffer) (KCl 0.3 M) at pH 9. As can be observed, the voltammogram contains a reversible couple (I_{pc}/I_{pa}) and an irreversible peak at approximately — 1350 mV. All compounds showed a similar voltammetric pattern which is the typical behavior for a nitro derivative in mixed medium [20], wherein the reversible couple corresponds to the one-electron reduction of the nitro group to form the corresponding $R–NO_2^{\bullet-}$, according to Eq. (1):

$$RNO_2 + e^- \leftrightarrows R–NO_2^{\bullet-} \quad (1)$$

and the irreversible peak corresponds to the further reduction of the nitro radical anion to the corresponding hydroxylamine derivative, according to Eq. (2):

$$R–NO_2^{\bullet-} + 3e + 4H^+ \rightarrow RNHOH + H_2O. \quad (2)$$

In this mixed medium the nitro/nitro radical anion couple was very well resolved at alkaline pHs (pH ≥ 9). By the adequate selection of the initial and switching potentials it is possible to work with the isolated couple in the voltammograms. In Fig. 3, the isolated $RNO_2/R–NO_2^{\bullet-}$ couple for the three synthesized nitrofuryl derivatives is shown. From these couples we have obtained ΔE_P values near 60 mV (Table 1) confirming the one electron character of the reduction. Furthermore, we have obtained a direct relation between the reduction potential and the electron withdrawing effect of the substituents in 3,5 positions on the dihydropyridine ring. When the 3,5 positions are both substituted with the strongest electron acceptor group, i.e. the CN group (compound FDHP2), the nitro group was easier reduced (Table 1). The peak potential values were slightly dependent of the sweep rate but the current ratio I_{pa}/I_{pc} was clearly dependent of the sweep rate pointing out to a coupled chemical reaction. From the

Table 1
Cathodic peak potentials (E_{PC}), disproportionation decay constants (k_2) and half-life times ($t_{1/2}$) for the nitro radical anion electrochemically formed in mixed medium, 60/40: DMF/citrate buffer (KCl 0.3 M), pH = 9

Compounds	$-E_{pc}$ (mV)	ΔE_p (mV)	k_2 (M^{-1} s^{-1})	$t_{1/2}$[a] (s)
FDHP1	760	66±3	4602±172	0.22±0.02
FDHP2	700	62±3	3491±133	0.26±0.03
FDHP3	721	61±2	2851±107	0.35±0.01

[a] Calculated for 1 mM.

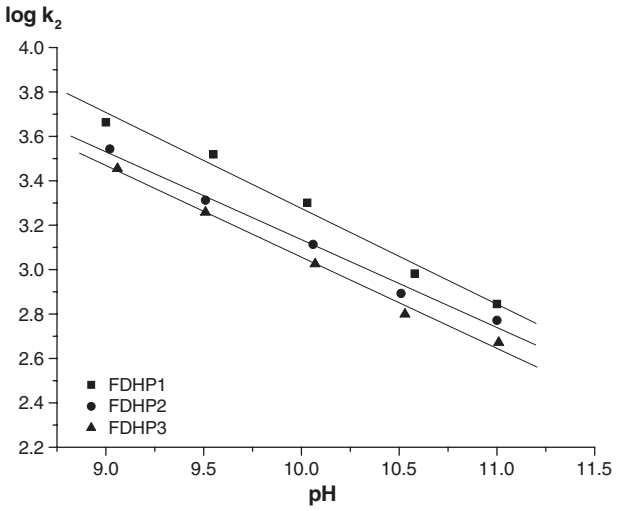


Fig. 4. Disproportionation rate constant dependence on pH for the nitro radical anion of 1 mM nitrofuryl derivatives in mixed medium, 60/40: DMF/citrate buffer (KCl 0.3 M).

dependence of the current ratio with the sweep rate (Fig. 3) we can deduce a mechanism involving an irreversible chemical reaction following the reversible one-electron reduction of RNO_2 for all the compounds (EC_{irrev} mechanism). This EC_{irrev} mechanism is evidenced by I_{pa}/I_{pc} values increasing and tending to 1 with the increase of sweep rate. Furthermore, the current ratio values were dependent of the concentration of the nitrofuryl derivative indicating a second order for the chemical reaction [21]. According to previous works, the coupled chemical reaction in mixed medium is a disproportionation reaction [22] and consequently we can obtain the disproportionation rate constants by applying the well known procedure described by Olmstead and Nicholson [18]. The occurrence of a father–son reaction as previously reported in non-aqueous medium [9] was not observed in this mixed medium. In Table 1, the decay rate constants for the disproportionation reaction (k_2) of the nitro radical anion formed from the nitrofuryl 1,4-DHP derivatives are shown. From these results it is possible to conclude that FDHP1 produced the nitro radical anion most hardly formed (highest reduction potential) but its generated radical was the most unstable (highest k_2). Furthermore we have found that the stability of the nitro radical anion is strongly pH-dependent. We have calculated the k_2 values and the corresponding half-life times for the nitro radical anion from all compounds at different pH values but all showed the same tendency. In Fig. 4 it is possible to observe a linear dependence between the logarithm of the disproportionation rate constant and pH. From the above linear dependence it is possible to obtain the following regression curves for FDHP1, FDHP2 and FDHP3, respectively:

$$\log k_2 = -0.44\ \mathrm{pH} + 7.65 (r^2 = 0.992)$$

$$\log k_2 = -0.39\ \mathrm{pH} + 7.11 (r^2 = 0.994)$$

$$\log k_2 = -0.47\ \mathrm{pH} + 7.72 (r^2 = 0.997)$$

By extrapolation, from the above equations, we have obtained k_2 values of 2.48, 1.67 and 1.75×10^4 M^{-1} s^{-1} for FDHP1, FDHP2 and FDHP3, at pH 7.4, respectively.

From the above results it is possible to affirm that the disproportionation reaction is pH-dependent obeying the following equation:

$$2\ \mathrm{R} - \mathrm{NO}_2^{\bullet -} + 2\mathrm{H}^+ \xrightarrow{k_2} \mathrm{RNO}_2 + \mathrm{RNO} + \mathrm{H_2O}. \quad (3)$$

In all cases the stability of the nitro radical anion was increased when the pH increased. This result is in accord with the above Eq. (3), i.e. when the proton concentration decreases the decay reaction is not favoured.

3.2. Non-aqueous medium

The evaluation of formation and stability of the nitro radical anion from all the studied nitrofuryl derivatives was also studied in a totally non-aqueous medium containing 0.1 M TBAP in 100% DMF . The one-electron reduction couple due to the nitro

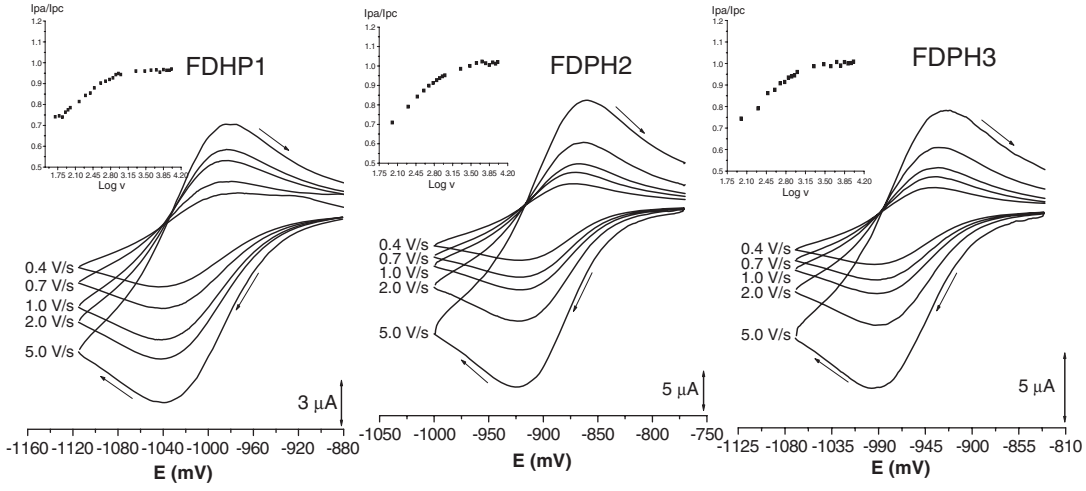


Fig. 5. Cyclic voltammograms of the electrochemically formed $R–NO_2/R–NO_2^{\bullet -}$ couple from 1 mM solution of FDHP1, FDHP2 and FDHP3 in non-aqueous medium at different sweep rates. Inset: dependence of the current ratio on sweep rate for each set of experiments.

radical anion formation was isolated for the three compounds. Fig. 5 shows cyclic voltammograms due to this couple at different sweep rates for all the nitrofuryl derivatives. In a similar way, to that occurring in mixed medium, this result is indicative of a coupled chemical reaction following the one-electron reduction of the nitro group. However, in the case of non-aqueous medium, previous studies have shown that this coupled chemical reaction would correspond to a dimerization instead of the disproportionation in mixed medium [6,7]. Using the theoretical approach of Olmstead et al. for a dimerization or disproportionation coupled reaction [18,19] we have calculated the second-order rate constant, (k_2), obtaining different values depending if disproportionation or dimerization was assumed. To decide which type of coupled reaction depicts better the real mechanism, a simulation procedure was used. The simulation procedure involved simulating cyclic voltammograms using alternatively either the obtained disproportionation or dimerization rate constant and then compares the simulated cyclic voltammograms with the experimental ones. As can be seen, in Fig. 6, there are a perfect fit between the experimental and simulated curves only for the case of dimerization confirming the $EC_{2,dim}$ mechanism and supporting the validity of the obtained constant values. The obtained values for the dimerization rate constants for each nitro radical anion derivative are shown in Table 2.

In contrast with the mixed medium, the nitro radical anion from FDHP1 required more energy for its formation but the radical was the most stable. Furthermore, it is important to remark that in non-aqueous medium a direct relation between the electron affinity of the compounds (reflected in its E_{pc} values) and the stability of the nitro radical anion existed. In Fig.

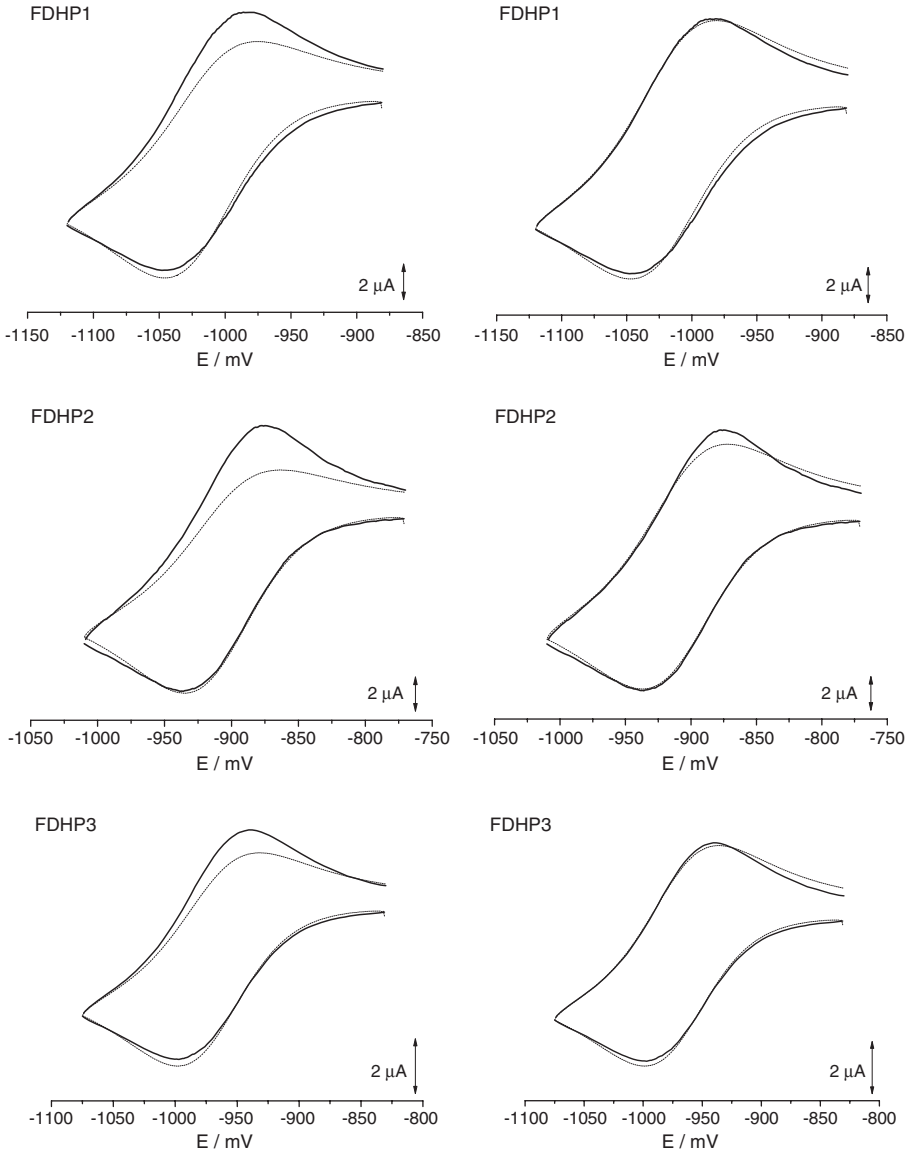


Fig. 6. Comparison of experimental (solid line) and simulated (dashed line) cyclic voltammograms of 1 mM solution of FDHP1, FDHP2 and FDHP3 in non-aqueous medium at 1 V/s considering either: disproportionation (left hand) or dimerization (right hand). Simulation considered an EC_2 mechanism wherein the parameters for the chemical step are: $k_{2,disp}$=3811, 6456, 4765 or $k_{2,dim}$=779, 1440, 1100 M^{-1} s^{-1}, for FDHP1, FDHP2 and FDHP3, respectively.

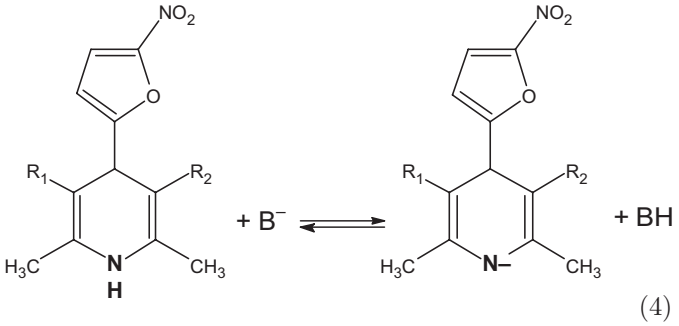
Table 2
Cathodic peak potentials (E_{pc}), dimerization decay constants (k_2) obtained in non-aqueous medium by cyclic voltammetry in, E_7^1 values for the RNO_2/ $R–NO_2^{\bullet-}$ couple and equilibrium constant for the electron transfer from $R–NO_2^{\bullet-}$ to oxygen (K_{O2}) for the $R–NO_2^{\bullet-}$ from nitrofuryl 1,4-DHP derivatives and some corresponding nitranion derivatives

Compound	$-E_{pc}$/mV vs Ag/AgCl	$-E_7^1$/mV vs NHE	k_2/M^{-1} s^{-1}	K_{O2}
FDHP1	1044	605	779±37	4.2×10^7
FDHP1[a]	1154	700	606±71	1.7×10^9
FDHP2	929	506	1440±68	8.9×10^5
FDHP3	998	566	1100±83	3.3×10^7
FDHP3[a]	1095	649	699±65	2.3×10^8
Nitrofurazone[b]	886	242	750±43	30
Nifurtimox[b]	930	286	486±36	166
Benznidazole[c]	1019	380	–	6400

[a] Indicates the nitranion form of the corresponding nitrofuryl derivative.
[b] Data obtained from Ref. [16].
[c] Data obtained from Ref. [22].

7, we can observe a linear relation between E_{pc} and $k_{2,dim}$ values, meaning that whereas it is most difficult to be reduced the nitrofuryl derivative which was more stable was the formed radical.

As was previously reported, these nitrofuryl 1,4-DHP derivatives [9], generated a stable nitranion derivative by reaction with a base (B^-, i.e. alcoholic Na OH in Ref. [9]) in non-aqueous medium, according to the following equation:



(4)

Furthermore, in that work, the nitranion also was generated by electrolysis in bulk. In that case, the nitro radical anion exhaustively generated by the electrolysis procedure acts as a base (B^- in Eq. (4)) thus generating the nitranion derivative. Therefore, when the base B^- corresponds to the electrogenerated nitro radical anion, Eq. (4) is a self-protonation reaction.

On the other hand, in the present study wherein only cyclic voltammetry experiments (short sweep condition) are involved, the nitranion derivative was not detected (Fig. 5). Consequently, in this condition, we discarded the occurrence of a self-protonation reaction with formation of a nitranion derivative as occurred in bulk electrolysis. Probably the change of reaction pathway on passage from bulk electrolysis to cyclic voltammetry is due to that in the case of bulk electrolysis the mass transport is facilitated, which results in an increase of the radical anion concentration acting as a base in the vicinity of the electrode thus promoting the occurrence of the self-protonation reaction according to Eq. (4).

Furthermore in order to examine the voltammetric behaviour by preventing any possibility of self-protonation we have added a small quantity of a proton donor as acetic acid. As can be observed in Fig. 8 no changes were appreciated after the addition, confirming that no self-protonation is involved in the cyclic voltammetric experiment.

Furthermore, in order to investigate if the reduction of the nitrofuryl moiety would be affected by the presence of the nitranion in the dihydropyridine moiety we have compared nitranion derivatives (quantitatively generated by reaction with base) with the corresponding parent compounds. In Fig. 9, we can observe the effect on the reduction of the nitro group resultant of changing the –NH bonding by the nitranion ($–N^-$) in the dihydropyridine moiety. As can be seen, all the compounds were more hardly reduced in its nitranion form increasing its cathodic peak potential in about 100 mV when passing from the dihydropyridine to the nitranion form. Therefore, the nitro radical anion was more easily formed from the nitrofuryl 1,4-DHP derivatives than the nitrofuryl nitranion derivatives. We have also calculated the stability of the formed nitro radical anion from the nitranion species by applying the same methodology that in the case of the dihydropyridine derivatives. As can be seen from the results in Table 2, the obtained nitro radical anions from the nitranion derivatives resulted to be a little more stable than the normal nitro anions. Summarizing, the negative charge on the nitranion moiety produced an extra energetic requirement for the nitro group reduction but the resulting nitro radical anion was more stable. By extrapolating these results to a biological condition we can speculate that a possible enzymatic reduction would be more difficult for the nitranion species.

The above data in aprotic medium not only described the situation in that medium but also permit to obtain biological significance parameters as the E_7^1 and K_{O2}. The E_7^1 value is a parameter that accounts the energy necessary to transfer

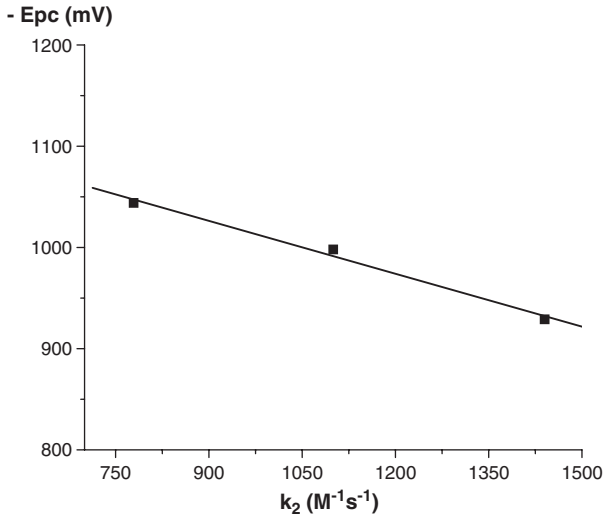


Fig. 7. Cathodic peak potential values versus dimerization rate constant values from the one-electron reduction of nitrofuryl derivatives in non-aqueous medium.

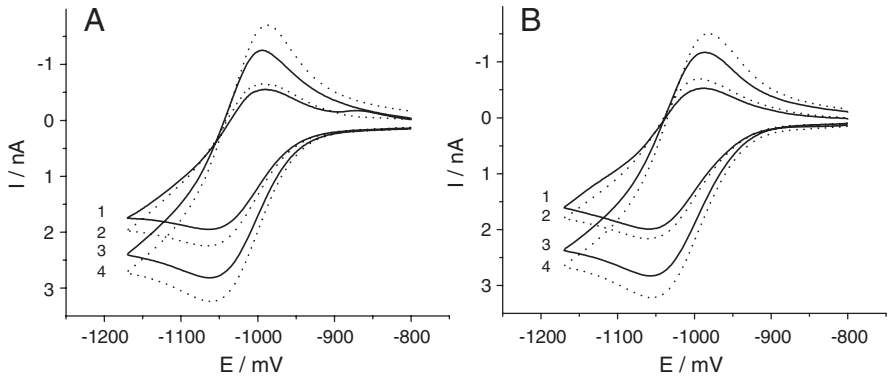


Fig. 8. Cyclic voltammograms of the electrochemically formed R–NO_2/ R–$NO_2^{\bullet-}$ couple from 1 mM solution of FDHP1 in non-aqueous medium without (A) and with (B) 0.04 mM of acetic acid. Sweep rates: 0.075 (1), 0.1 (2), 0.2 (3) and 0.3 (4) V/s.

the first electron to an electroactive group to form a radical anion, at pH 7 in aqueous medium, and it is considered as indicative of nitro radical anion formation in vivo [22]. Furthermore, a correlation between the cathodic peak potential (E_{pc}), obtained in aprotic media, with the E_7^1 values obtained by pulse radiolysis, has been previously described [23]. In fact, from that paper a linear relation between E_7^1 and E_{pc} was revealed. Consequently, with the obtained experimental values of E_{PC} in aprotic medium we calculated the E_7^1 values for all the studied compounds by interpolating in the corresponding straight line. The E_7^1 values of the RNO_2/R–$NO_2^{\bullet-}$ couple may be used to assess not only the thermodynamic feasibility of one-electron reduction of RNO_2 by any possible "nitroreductase" but also the likelihood of electron donation from the R–$NO_2^{\bullet-}$ to oxygen in the well-known "futile reduction" present in aerobic conditions:

$$R - NO_2^{\bullet-} + O_2 \overset{K_{O2}}{\cdot} RNO_2 + O_2^{\bullet} \tag{5}$$

In order to obtain the equilibrium constant K_{O2}, from Eq. (5), we have used the well-known thermodynamic relation:

$$\Delta E = RT/nF \ \ln K \tag{6}$$

where, in this case, at 25 °C:

$$0.059 \log K_{O_2} = \{-0.155 - [E_{(RNO_2/RNO_2^{\bullet})}]\} \tag{7}$$

where −0.155 V is the redox potential for the couple $O_2/O_2^{\bullet}$ in the nonstandard condition of 1 mM (the same condition

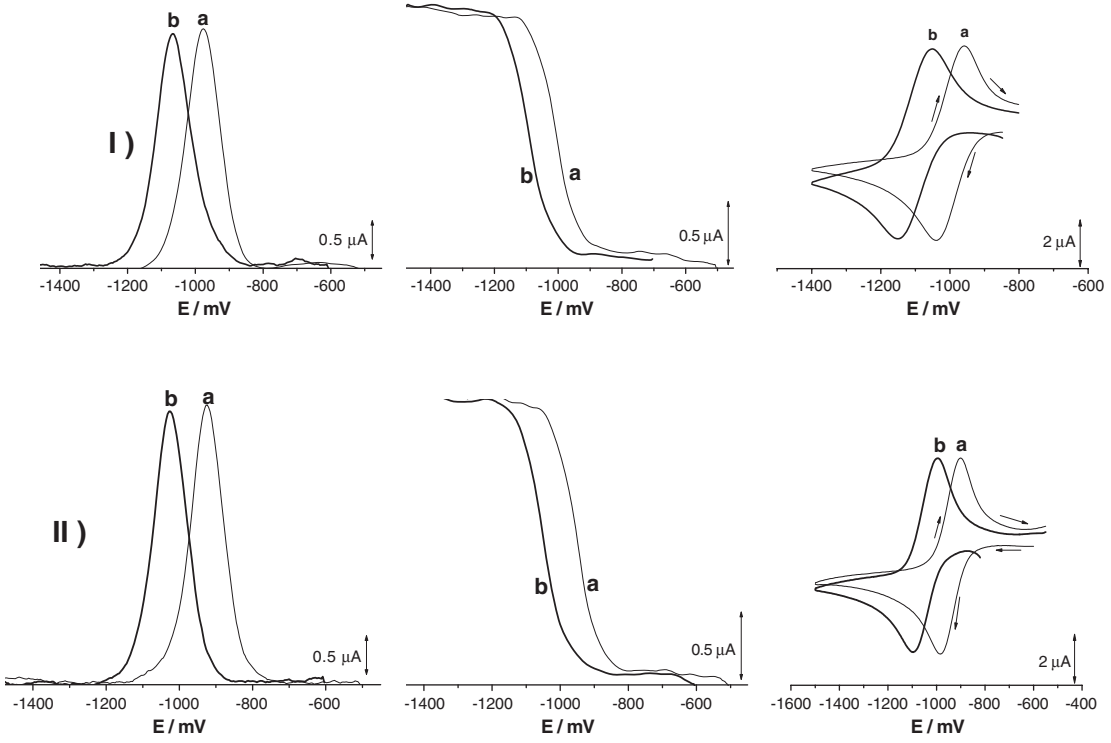


Fig. 9. Differential pulse and tast polarograms, and cyclic voltammograms of the one electron nitro reduction in a nitrofuryl 1,4-DHP derivative containing: (a) the dihydropyridine moiety and (b) the nitranion moiety. I) FDHP1 and II) FDHP3.

as $RNO_2/R–NO_2^{\bullet -}$) in aqueous medium, pH 7 [22] and $[E_{(RNO_2/RNO_2^{\bullet})}]$ corresponds to the E_7^1 value.

Table 2 lists the calculated values of E_7^1 and K_{O2} for the $R–NO_2^{\bullet -}$ of all the studied nitrofuryl 1,4-DHP derivatives and its comparison with well recognized drugs that acts via $R–NO_2^{\bullet -}$. From these results we can assume that the $R–NO_2^{\bullet -}$ from the new synthesized nitrofuryl 1,4-DHP, FDHP2, could be biotransformated via enzymatic reduction, because the energetic of formation (E_7^1) is lower or comparable with that obtained for the well-known enzymatic reducible drugs such as nitrofurazone, nifurtimox and benznidazole. From the obtained K_{O2} values, we can assume that the nitro radical anion generated from the compound FDHP2 shows a similar ability to carry out the "futile" reduction than the current drug nifurtimox. Consequently, this possible new antichagasic compound shows a good thermodynamic approach to produce redox cycling. On the other hand, the higher decomposition rate constant of the radical from FDHP2 (k_2 value) represents an advantage of FDHP2 respect of nifurtimox due to the minor residence in the host, probably with a lower incidence of toxic reactions.

4. Conclusions

According to the above results, it is clear that from this type of compounds we can modulate both, the reduction ability of the nitrofuryl group and the stability of the nitro radical anion according to the electronic characteristics of the dihydropyridine ring. In fact, an excess of negative charge in the dihydropyridine ring, as in the case of the nitranion, produced an increase of the reduction potential making more difficult a possible enzymatic reduction. On the other hand, the presence of electron-acceptor substituents produce a decrease of the electron density on the dihydropyridine ring, thus diminishing the electron density on the nitrofuryl moiety producing compounds more easily reducible and consequently capable to be enzymatically reduced.

From the voltammetric measurements it is possible to conclude that the nitro radical anion from FDHP2 could be biologically generated via enzymatic reduction, because the energetic of its formation is similar with that obtained for other well-known enzymatic reducible drugs as nifurtimox and benznidazole (both nitrocompounds used for the treatment of Chagas' disease). Furthermore, the higher decay constant of the nitro radical from FDHP2 could represent an advantage of this compound due to the minor permanence in the host with consequently lower toxicity.

We have shown that using cyclic voltammetric experiments it is possible to calculate parameters such as E_7^1, k_2 and K_{O2} providing simple diagnostic criteria to select in a first screening test drug with adequate biological performance.

Acknowledgements

The authors are grateful to FONDECYT (Research grant N° 8000016), Scholarship DAAD (Deustcher Akademisher Austauschdienst) and Universidad de Chile for financial support.

References

[1] A. Moncayo, Chagas' disease, Tropical Disease Research: Progress 1991–1992, WHO, Geneva, 1993, pp. 67–76.
[2] M. Dubin, S. Moreno, E. Martino, R. Docampo, A. Stoppani, Increased biliary secretion and loss of hepatic glutathione in rat liver after nifurtimox treatment, Biochem. Pharmacol. 32 (1983) 583–587.
[3] J. Maya, S. Bollo, L.J. Núñez-Vergara, J.A. Squella, Y. Repetto, A. Morello, J. Périé, G. Chauvière, *Trypanosoma cruzi*: effect and Mode of action of nitroimidazole and nitrofurane derivatives, Biochem. Pharmacol. 65 (2003) 999–1006.
[4] C.R. De Castro, E.G.D. De Toranzo, A.S. Bernacchi, M. Carbone, J.A. Castro, Ultrastructural alterations in ovaries from nifurtimox or benznidazole-treated rats—their relation to ovarian nitroreductive biotransformation of both drugs, Exp. Mol. Pathol. 50 (1989) 385–397.
[5] N. Gorla, O. Ledesma, G. Barbieri, I. Larripa, Thirteenfold increase of chromosomal aberrations non-randomly distributed in chagasic children treated with nifurtimox, Mutat. Res. 224 (1989) 263–267.
[6] C. Yáñez, J. Pezoa, M. Rodríguez, L.J. Núñez-Vergara, J.A. Squella, Voltammetric behavior of a 4-nitroimidazole derivative nitro radical anion formation and stability, J. Electrochem. Soc. 152 (2005) 46–51.
[7] S. Bollo, L.J. Núñez-Vergara, J.A. Squella, Cyclic voltammetric determination of free radical species from nitroimidazopyran: a new antituberculosis agent, J. Electroanal. Chem. 562 (2004) 9–14.
[8] F. Nesslany, S. Brugier, M. Mouriès, F. Le Curieux, D. Marzin, In vitro and in vivo chromosomal aberrations induced by megazol, Mutat. Res. 560 (2004) 147–158.
[9] J. Argüello, L.J. Núñez-Vergara, J.A. Squella, Electrogeneration of nitranion species from nitrofuryl substituted 1,4-dihydropyridine derivatives, Electrochem. Commun. 7 (2005) 53–57.
[10] G. Aguirre, E. Cabrera, H. Cerecetto, R. Di Maio, M. Gonzalez, G. Seoane, A. Duffaut, A. Denicola, M.J. Gil, V. Martinez-Merino, Design, synthesis and biological evaluation of new potent 5-nitrofuryl derivatives as anti-*Trypanosoma cruzi* agents. Studies of trypanothione binding site of trypanothione reductase as target for rational design, Eur. J. Med. Chem. 39 (2004) 421–431.
[11] G. Aguirre, L. Boiani, H. Cerecetto, M. Fernandez, M. Gonzalez, A. Denicola, L. Otero, D. Gambino, C. Rigol, C. Olea-Azar, M. Faundez, In vitro activity and mechanism of action against the protozoan parasite *Trypanosoma cruzi* of 5-nitrofuryl containing thiosemicarbazones, Biorg. Med. Chem. 12 (2004) 4885–4893.
[12] J. Stradins, S. Hiller, R. Gavars, G. Reihmanis, L. Baumane, in: G. Milazzo, P. Jones, L. Rampazo (Eds.), Biological Aspects of Electrochemistry, Birkhäuser, Basel, 1971, pp. 607–617.
[13] J. Tocher, D. Edwards, Electrochemical characteristics of nitrheterocyclic compounds of biological interest: I. The influence of solvent, Free Radic. Res. Commun. 4 (1988) 269–276.
[14] J. Tocher, D. Edwards, Electrochemical characteristics of nitrheterocyclic compounds of biological interest. V. Measurements and comparison of nitro radical lifetimes, Int. J. Radiat. Biol. 57 (1990) 45–53.
[15] T. Symons, J. Tocher, D. Tocher, D. Edwards, Electrochemical studies of nitroheterocyclic compounds of biological interest: VII. Effect of electrode material, Free Radic. Res. Commun. 14 (1991) 33–40.
[16] S. Bollo, L.J. Núñez-Vergara, C. Martinez, G. Chauvière, J. Périé, J.A. Squella, Voltammetric study of nitro radical anion generated from some nitrofuran compounds of pharmacological significance, Electroanalysis 15 (2003) 19–25.
[17] R.S. Nicholson, Semiempirical procedure for measuring with stationary electrode polarography rates of chemical reactions involving the product of electron transfer, Anal. Chem. 38 (1966) 1406.
[18] M.L. Olmstead, R.S. Nicholson, Cyclic voltammetry theory for the disproportionation reaction and spherical diffusion, Anal. Chem. 41 (1969) 862–864.

[19] M.L. Olmstead, R.G. Hamilton, R.S. Nicholson, Theory of cyclic voltammetry for a dimerization reaction initiated electrochemically, Anal. Chem. 41 (1969) 260–266.
[20] J.A. Squella, S. Bollo, L.J. Núñez-Vergara, Recent developments in the electrochemistry of some nitro compounds of biological significance, Curr. Org. Chem. 9 (2005) 565–581.
[21] G. Bontempelli, F. Magno, M. Mazzocchin, R. Seeber, Linear sweep and cyclic voltammetry, Ann. Chim. 79 (1989) 103.
[22] P. Wardman, Some reactions and properties of nitro radical-anions important in biology and medicine, Environ. Health Perspect. 64 (1985) 309320.
[23] A. Breccia, G. Berrilli, S. Roffia, Chemical radiosensitization of hypoxic cells and redoc potentials. Correlation of voltammetric results with pulse radiolysis data of nitro-compounds and radiosensitizers, Int. J. Radiat. Biol. 36 (1979) 85–89.