Voltammetric Study of Nitro Radical Anion Generated from Some Nitrofuran Compounds of Pharmacological Significance

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Abstract
The electrochemical behavior of 2-(5-amino- 1,3,4-oxadiazolyl)-5-nitrofuran (NF359) and its comparison with well-known drugs such as nifurtimox (NFX) and nitrofurazone (NFZ) in protic, mixed and aprotic media by cyclic voltammetry, tast and differential pulse polarography was studied. All the compounds were electrochemically reducible in all media being the reduction of the nitrofuran group the main voltammetric signal. The one-electron reduction couple due to the nitro radical anion formation was visualized in mixed (for NF359 and NFZ) and aprotic media (for all compounds). By applying a cyclic voltammetric methodology we have calculated the decay constants (k_2) of the corresponding nitro radical anions in mixed and aprotic media. In mixed medium data fit well with a disproportionation reaction of the nitro radical anion but in aprotic medium fit better with a dimerization reaction. Also, considering cyclic voltammetric measurements in aprotic media we have estimated the reduction potential of the $RNO_2/RNO_2^{\bullet-}$ couple in aqueous medium, pH 7 ($E^1{}_7$ values) finding very good correlation with $E^1{}_7$ values obtained by pulse radiolysis. Furthermore we have calculated the equilibrium constants from the electron transfer from nitro radical anion to oxygen (k_{O2}) finding that nitro radical anion from NF359 is thermodynamically favored to react with oxygen in respect to both NFZ and NFX.

Keywords: Nitrofuran derivatives, Voltammetric reduction, Antichagasic drug

1. Introduction

Nitroheterocyclic helpfulness in treatment of infectious diseases is a well-established fact. Mainly, 5-nitrofurans and 5-nitroimidazoles are the nitroheterocyclic drugs most used as antibacterial, antiprotozoal and anticancer agents [1 – 4]. But, it is also well established that these molecules present several adverse effects associated with its intake [5, 6].

Both pharmacological and toxic effects, are mediated by cytotoxicity and depend on the in vivo reduction of the nitro group, producing the nitro radical anion ($ArNO_2^{\bullet-}$) specie, which subsequently could produce more reactive species derived from oxygen by a specific redox cycling; or produce itself the damage by interactions with DNA [7 – 9].

Electrochemical studies of these nitro compounds are very relevant in order to characterize the redox activation that implies the one electron transfer to nitro group to produce nitro radical anion.

However, generally these studies are restricted only to obtain the reduction potential of the $ArNO_2/ArNO_2^{\bullet-}$ couple, because this parameter is well recognized as a very appropriate index to define the type of biological properties of the different nitrocompounds. Moreover, studies carried out by Olive [10] on a series of nitroheterocyclic compounds demonstrated the existence of a direct correlation among the polarographic half-wave potential ($E_{1/2}$) and the nitro-reduction rate in different biological systems. As was concluded in that work drugs with higher electroaffinity (smaller reduction potential) are generally the most toxic and mutagenic, being also characterized to be those metabolized more quickly. Furthermore there are a lot of other studies [11 – 13] dealing with relationship between reduction potentials and pharmacological activity showing that this parameter is of interest from electrochemical and pharmacological points of view.

On the other hand, it is generally accepted that the redox properties of the one-electron couple $ArNO_2/ArNO_2^{\bullet-}$ provide only a limited approach on the biological properties of compounds that requires redox activation. In order to obtain a more complete relationship between in vitro behavior of the molecules and physiological activity, many others factors as stereochemistry, lipid solubility, diffusion, enzymatic site binding, electron transfer reactions and kinetics also must be considered [7].

Trypanosomiasis is one of the major third world diseases, with several millions of new infections confirmed annually. *Trypanosoma cruzi* is the etiologic agent of Chagas' disease. The current chemotherapy against this disease is still inadequate and therefore efforts to find antichagasic drugs are of great importance. The main drug in use against Chagas' disease is nifurtimox, a 5-nitrofurfural derivative, but its use is associated with a high incidence of serious side effects [14]. On the other hand, nitrofurazone also has shown a very interesting trypanocidal activity [15, 16].

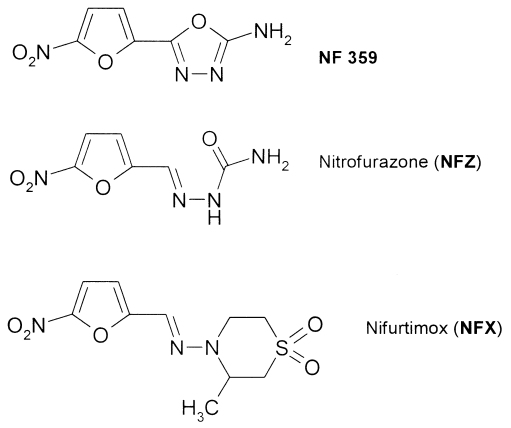

Fig. 1. Chemical structure of NF359, nitrofurazone and nifurtimox.

In the scope of our investigations tending to find new therapeutic alternatives for the Chagas' disease, we have synthesized a nitrofuran derivative (NF 359, Figure 1) very related to nitrofurazone, showing preliminary antichagasic activity. In this study we note the electrochemical characteristics of NF 359 focused mainly to the feasibility of obtaining stable nitro radical anions. Furthermore we reveal a comparative study within NF359 and a couple of well-known nitrofurans such as nifurtimox (NFX) and nitrofurazone (NFZ). There are a lot of articles wherein the electrochemistry of nitrofurans has been touched. In fact, the reduction potentials of nitrofurans were examined in the late 1960's using DC polarography [17]. Later on the ninety plus works related to the cyclic voltammetric behavior of several nitrofurans derivatives such as nitrofurazone, nifuroxime, nitrofurantoin and furazolidone [18–20] have been published. More recently the nitro radical anion formation from nifurtimox and nifuroxazide [21, 22] and the interaction of the nitro radical anion from nitrofurazone have been voltammetrically approached [23].

2. Experimental

2.1. Synthesis of NF 359

Synthesis of NF 359 (2-(5-amino-1,3,4-oxadiazolyl)-5-nitrofurane)2-carbonitrile-5-nitrofurane (700 mg, 5 mM) and hydrochloride semicarbazide (560 mg, 5 mM) were agitated in refluxed trifluoroacetic acid (20 mL) for 15 minutes; the cold solution (0 °C) was then carefully neutralized with sodium hydrogenocarbonate, the orange solid obtained was filtrated, washed with water and desiccated in vacuum giving NF359 (450 mg, 45% yield).

^{1}H NMR (D6 DMSO, 80 MHz) 6.33(s, NH2) 7.30 (d, H4) 7.80 (d, H3)

^{13}C NMR (D6 DMSO, 50 MHz) 111.3 (C3) 114.9 (C4) 133.5 (C2) 150.6 (C5) 150.8 (C′2) 157.0 (C′5)

Mass spectrometry (DCI/NH_3) m/z 231 ($M^+ + 2NH_3$,100)

2.2. Reagents and Solutions

NF 359 was synthesized and characterized by one of us. Nitrofurazone (NFZ) and Nifurtimox were commercially obtained from Sigma Aldrich and Laboratorios ROCHE Quimicos e Farmaceuticos S. A., Brasil, respectively. 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 to obtain final concentrations of 0.1 and 1 mM, respectively. A mixture of 30/70 : ethanol/Britton-Robinson buffer (KCl 0.3 M) for a protic medium and a mixture 60/40 : DMF/citrate buffer (KCl 0.3 M) for a mixed medium were used as reaction media. The pH was adjusted with little aliquots of concentrated NaOH or HCl, respectively.

In mixed medium the pH measurements were corrected according to the following equation [24]:

$pH^* - B = \log U^{\circ}_{H}$ were pH* equals $-\log a_H$ in the mixed solvent, B is the pH meter reading and the term $\log U^{\circ}_{H}$ is the correction factor for the glass electrode, which was calculated from the different mixtures of DMF and aqueous solvent, according to a previously reported procedure [25].

Dimethylformamide (DMF) and tetrabutyl ammonium iodide (TBAI) as solvent and supporting electrolyte were used in aprotic media.

2.3. 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 platinum wire as a counter electrode were used. All potentials were measured against Ag/AgCl 3 M.

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

For the kinetic analysis carried out in mixed and aprotic media, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible one electron couple ($ArNO_2/ArNO_2^{\bullet -}$) was measured for each individual cyclic voltammogram according to the procedure described by Nicholson [26]. The scan rate was varied between 0.1 and 10 V/s.

Using the theoretical approach of Olmstead et al. for dimerization or disproportionation [27, 28], 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 rate constant, drug concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by the equation

$$\omega = k_2 \times C_o \times \tau$$

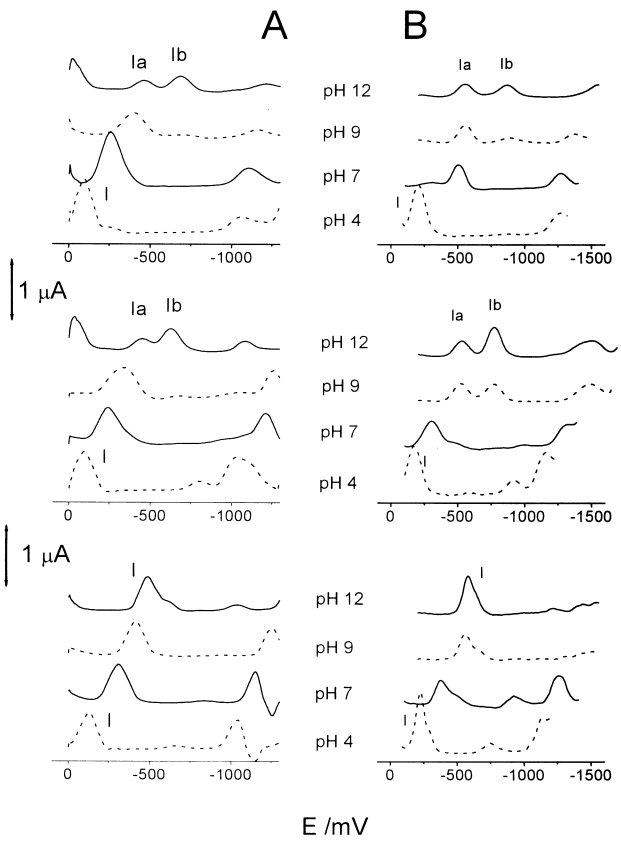

Fig. 2. Differential pulse polarogram at different pH values of 0.1 mM of NF 359, NFZ and NFX in A) protic medium, 30/70:ethanol/Britton-Robinson buffer (KCl 0.3 M) and B) mixed medium, 60/40:DMF/citrate buffer (KCl 0.3 M)

Where k_2 is the second-order rate constant for the decomposition of $ArNO_2^{\bullet-}$, C_o is the nitrocompound concentration and $\tau = (E_\lambda - E_{1/2})/v$. Consequently we can obtain the second order rate constant for the decomposition of the nitro radical anion from the slope of the straight line ω versus τ. The assumption that the decomposition of $ArNO_2^{\bullet-}$ follows second-order kinetics is supported by the obtained linearity between the kinetic parameters ω and τ.

3. Results and Discussion

Nifurtimox (NFX), nitrofurazone (NFZ) and NF 359 were electrochemically reduced at mercury electrodes (DME and HMDE) in protic, mixed and aprotic media. According to our previous works [21, 29] and the literature [19], the most easily electroreducible group in the molecules is the nitro group. However, the reduction of the imine moiety present in the structures also is possible, but at higher potential than the nitro group [30].

3.1. Protic Medium

As a protic medium we have used a mixture of 30/70:ethanol/Britton-Robinson buffer (KCl 0.3 M). In this medium all the compounds were reduced, producing well-resolved signals. Figure 2A, shows DP polarograms obtained at different pHs (4, 7, 9 and 12), which gives only one-reduction peak (peak I) under – 1 V in acidic and neutral pH for all the nitrofuran derivatives studied. Above pH 9, NFZ and NF 359 show a splitting of peak I in two new ones (peaks Ia and Ib), while for NFX the above splitting is only insinuated.

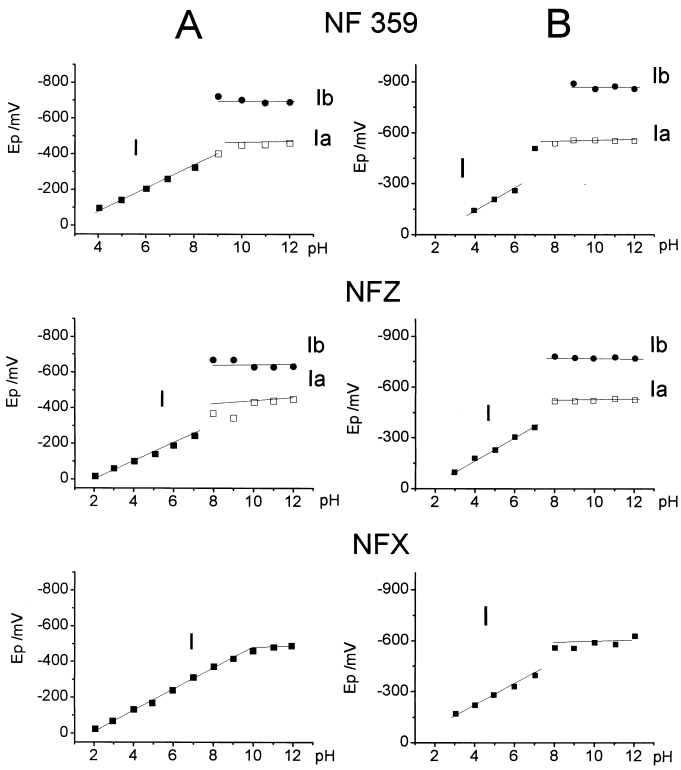

Fig. 3. Peak potential dependence with pH for the nitro reduction of nitrofurans derivatives by DPP, in A) protic medium and B) mixed medium.

In accord with the literature [30], the electroreduction of the imine group appeared at sufficiently higher potential than the nitro group, without interferences between them. We have focused our attention only on the nitro reduction in order to evaluate the influence of the substitution in position 5.

In order to study the pH influence on the nitro reduction we have evaluated the behavior of the peak potential, (E_p) obtained by DPP, and the limiting current (I_{lim}) obtained by Tast polarography, at different pH between 2 and 12. In Figure 3A, the E_p vs. pH plots show that the peak potential of signal I is pH-dependent shifting to more negative potentials when pH increased (2 to 10). The slope values for E_p/pH plots were 60.8, 42.4 and 58.1 mV/pH for NF 359, NFZ and NFX, respectively. But up to pH 10 the peak potential remained constant for all the compounds. Concomitant with this pH independence of the signal I, a new signal (Ib) for NF359 (up to pH 9) and NFZ (up to pH 8) was observed. This new signal corresponded to the splitting of signal I. The peak potentials of new signals (Ia and Ib) are totally pH-independent.

The limiting currents remain practically pH independent between pH 2 – 8, according to a diffusion controlled process (data not shown). The limiting current values obtained at the same concentration, at pH 4 and 7, for each nitro compound were very close, then we can conclude that the electroreduction of the three nitrofurans consume the same number of electrons. As the process for the nitroreduction of NFX and NFZ is well described as a four-electron process [21, 31], we can conclude that in the case of NF359 it also involves a 4-electron reduction process to form the corresponding hydroxylamine according to the well-known Equation 1:

$$ArNO_2 + 4e^- + 4\,H^+ \rightarrow ArNHOH + H_2O \quad (1)$$

On the other hand, beyond to pH 8, NFZ and NF359 change its reduction mechanism and two new signals were produced. This behavior also has been described in the literature for the case of NFZ [32], and was attributed to the following mechanism in two separate steps:

$$ArNO_2 + e^- \rightleftharpoons ArNO_2^{\bullet -} \quad (2)$$

$$ArNO_2^{\bullet -} + 3\,e^- + 4\,H^+ \rightarrow ArNHOH + H_2O \quad (3)$$

By comparison of the peak potential values for the nitroreduction, data shows that NFX nitroreduction occurs at more cathodic potentials than the others two compounds, in all the pH range, e.g., at pH 7 E_p values were – 312, – 244 and – 260 mV for NFX, NFZ and NF359, respectively. This can be attributed to the electronic effect of the substituents in position 5, which in NFZ and NF359 are more electron withdrawing than 5-substituent of NFX, which produce a lower peak potential of NFZ and NF359.

The characterization of the cyclic voltammetric behavior in protic medium of all the compounds at different pH (3, 6, 9, 12) also was carried out. As shown in Figure 4, there are concordance between the polarographic and cyclic voltammetric signals, i.e., one main cathodic peak (I) due to the four electron reduction of the nitro group, and in strong alkaline media we observed the splitting of this signal in two new peaks I a and I b. In the case of NFX the splitting was not observed.

The sharp character of the cyclic voltammetric peaks reveals an adsorption phenomenon involved in the reduction process. This was corroborated by logarithmic analysis, where the slope of log I_p vs. log v plot gave values of 0.64, 0.68 and 0.62 for NFZ, NF 359 and NFX, respectively. Then the process of nitroreduction is a mixture of diffusion-adsorption phenomena.

Summarizing, in protic medium, NFX was the most difficult nitrofuran derivative to be reduced and its nitro radical anion signal was not possible to be observed in isolated form. On the other hand the nitro radical anion signals were observed for NFZ and NF359. These signals were not applied in this report because: a) its limiting currents were adsorption-diffusion mixed and b) the nitro radical anion was not sufficiently stabilized to produce a couple in the cyclic voltammetric experiment.

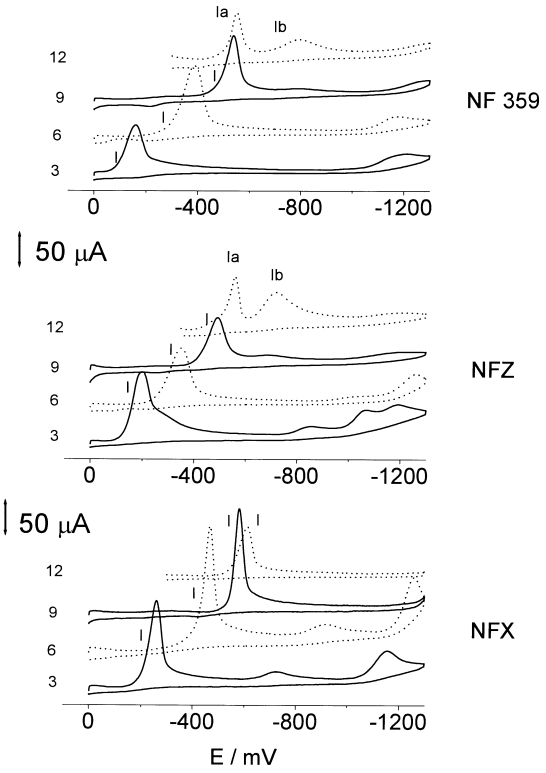

Fig. 4. Cyclic voltammograms of all nitrofurans derivatives (5 mM solution) at different pHs in protic medium, 30/70: ethanol/Britton-Robinson buffer (KCl 0.3 M). Sweep rate: 1 V/s.

3.2. Mixed Medium

Figure 2B shows the DP polarograms in mixed medium (60/40: DMF/citrate buffer (KCl 0.3 M)) at different pHs, 4, 7, 9 and 12. We can observe a relatively similar behavior than in the protic medium, wherein between pH 2 and pH 8 only one signal due to the 4-electron reduction is shown. At alkaline pHs the splitting of this signal, with the isolated nitro radical anion formation signal (I a) was observed for NFZ and NF 359. On the other hand, NFX did not produce separate signals for the nitro group, in all the pH range (2 – 12) and remain with its 4-electron, 4-proton mechanism. However in a mixed media containing 0.1 M tetrabutylammonium iodide as supporting electrolyte was possible to separate the signal due to the nitro radical anion formation for NFX.

Figure 3B shows the peak potential behavior of the nitro reduction signal with pH, showing the existence of two different zones. The first zone, between pH 2 and 7, is pH-dependent, with slopes values for E_p/pH plot of 57.8, 49.3 and 55.4 mV/pH for NF 359, NFZ and NFX, respectively. A second zone, at pH > 7, is pH-independent. The limiting currents remain practically pH-independent between pHs 2 – 7 according to a diffusion controlled process. Moreover the I_{lim} values for the three compounds were very close (data not shown), then we can assume, in a similar way as in protic media, that the main signal is also due to a four-electron reduction.

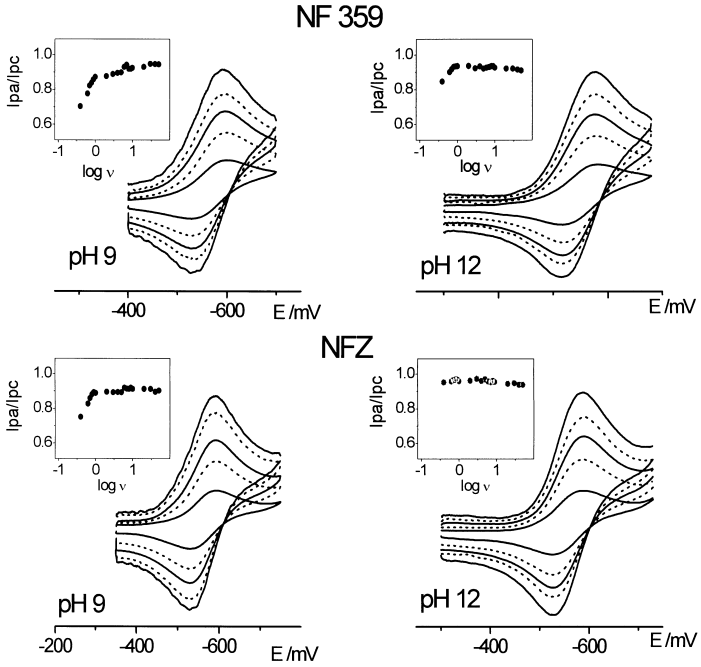

Fig. 5. Cyclic voltammograms of the isolated couple $ArNO_2$/$ArNO_2^{\bullet -}$ of NF359 and NFZ (5 mM solution) in mixed medium (DMF/Citrate : 60/40 0.3 M KCl) at pH 9 and 12. Sweep rates: 0.1, 0.5, 1, 5 and 10 V/s. Inset: Current ratio, I_{pa}/I_{pc}, dependence on sweep rate for the same experiment.

From the comparison of peak potential values for the nitroreduction at three different pHs, we can conclude that in this medium, the nitro group of NFX is more difficult to be reduced than the others two compounds, probably a consequence of the lower electro withdrawing effect of the substituents in position 5.

From the cyclic voltammetric experiment we can appreciate a similar behavior to the observed in protic media but without the adsorptive type interferences in the shape of the voltammograms. In fact, in mixed medium, we have obtained a 0.5 value for the slope of log I_p vs. log v plot, indicating a pure diffusion control of the electrode process for all the studied nitrofuran derivatives. Probably in mixed media the organic co-solvent displace adsorbed species from the electrode surface avoiding the adsorptive type interferences observed in protic medium.

Another important difference observed in mixed medium respect to the protic medium is that the one-electron couple due to the nitro radical anion formation is perfectly isolated in the case of NF359 and NFZ. In Figure 5, the cyclic voltammograms of both compounds, at pH 9 and 12, for the isolated $ArNO_2/ArNO_2^{\bullet -}$ couple at different sweep rate are shown. From these voltammograms we can obtain the following information: a) ΔE_p was ≈ 60 mV for all sweep rates studied (0.1 – 10 V/s), then the 1-electron transfer and nitro radical anion formation was verified, b) peak potentials were sweep rate independent consistently with a reversible electron transfer process. c) The current ratio (I_{pa}/I_{pc}) vs. sweep rate plot (insets Figure 5) reveals an ECi mechanism for both nitrofuran derivatives at pH 9 and only for NF359 at pH 12. An ECi mechanism implies a chemical reaction that occurs after the electron transfer and is evidenced by I_{pa}/I_{pc} values increasing and tending to 1 with the increase of sweep rate. d) Also, a second order for this chemical reaction was determined from the observed dependence of I_{pa}/I_{pc} with the nitrofuran derivatives concentration (data not show). In the case of first order chemical reaction, the current ratio is concentration independent.

According to the literature information [9], this chemical reaction represents the decay of nitro radical anion electrochemically formed and corresponds to a disproportionation reaction:

$$ArNO_2 + e^- \rightleftharpoons ArNO_2^{\bullet -} \quad (4)$$

$$2\ ArNO_2^{\bullet -} + 2\ H^+ \rightarrow ArNO_2 + ArNO + H_2O \quad (5)$$

According to a previously informed methodology [27] we have obtained the decay rate constant (k_{disp}). The kinetic decay constants (k_{disp}) calculated for each condition were: (4100 ± 325) $[Ms]^{-1}$ and (1200 ± 243) $[Ms]^{-1}$ for NF359 and NFZ at pH 9, respectively and (1570 ± 213) $[Ms]^{-1}$ for NF359 at pH 12.

On the other hand, nitro radical anion of nitrofurazone at pH 12 is sufficiently stabilized showing a response in accord with a reversible electron transfer without coupled chemical reactions, i.e., the nitro radical anion did not decay in this experimental condition.

For nifurtimox, the splitting of the 4-electron signal was slightly observed like a shoulder in the voltammetric peak, and then a study of nitro radical anion formation from nifurtimox in this mixed media was impossible.

3.3. Aprotic Medium

In order to complete the electrochemical characterization of the nitro radical anion from the nitrofuran derivatives, a totally aprotic reaction medium like 100% DMF and TBAI as supporting electrolyte was used. Furthermore the study in this medium resembles in some way the lipophilic environment, which is found in biological membranes.

The isolated $ArNO_2/ArNO_2^{\bullet -}$ couples for the three nitrofurans derivatives were possible to be obtained (data not shown), included for NFX. In this reaction media, NFZ is the most easily reducible nitrofuran derivative ($E_{pc} = -886$ mV), and NFX and NF359 there have no significative differences between them (-930 and -942 mV, respectively).

From the I_{pa}/I_{pc} evolution with the sweep rate of the experiment (Figure 6) an ECi mechanism can be assumed. However, according to previous electrochemical studies, the most probable decay reaction for the nitro radical anion in a totally aprotic medium is not the disproportionation but a dimerization reaction [33]. From this conclusion and applying the corresponding curves for dimerization decay [28] we calculated the decay constant obtaining the following

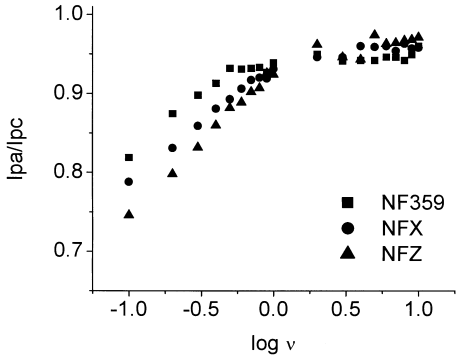

Fig. 6. Current ratio, I_{pa}/I_{pc}, dependence on sweep rate for the cyclic voltammograms of the isolated couple $ArNO_2/ArNO_2^{\bullet -}$ of NF359, NFZ and NFX (5 mM solution) in aprotic media (100% DMF ITBA 0.1 M).

values: (305 ± 50) $[Ms]^{-1}$; (750 ± 43) $[Ms]^{-1}$ and (486 ± 36) $[Ms]^{-1}$ for NF359, NFZ and NFX, respectively. These results indicate that the NFZ radical anion is more unstable than the others two radicals in aprotic media which implies a higher deactivation and lower half-life. On the other hand there are no significant differences in the stability of radicals generated from NF359 and NFX.

The above-obtained data in aprotic medium for peak potential of nitro radical anion formation describe the ability of the nitrofurans derivatives to form the nitro radical anion in this medium. However, as has been previously described there exists a good correlation between the cathodic peak potential, obtained in aprotic media, with the E_7^1 value obtained by pulse radiolysis [34]. The E_7^1 value is a parameter that accounts for the energy necessary to transfer the first electron to an electroactive group, at pH 7 in aqueous medium, to form a radical anion and it is considered as indicative of nitro radical anion formation in vivo. Consequently with the experimental obtained values in aprotic medium we can calculate the E_7^1 value for all the studied compounds. Table 1 lists the peak potential obtained by cyclic voltammetry. The calculated E_7^1 from electrochemical measurements, and the described E_7^1, from pulse radiolysis, show good correlation between them.

The potential peak values of the $ArNO_2/ArNO_2^{\bullet -}$ couple may be used to assess not only the thermodynamic feasibility of one-electron reduction of $ArNO_2$ by any possible "nitroreductase" but also the likelihood of electron donation from the nitro radical anion to potential acceptors.

Table 1. E_7^1 values for the $ArNO_2/ArNO_2^{\bullet -}$ couple and equilibrium constant for the electron transfer from $ArNO_2^{\bullet -}$ to oxygen.

	E_{pc} (mV)	Voltammetric calculated E_7^1 (mV)	E_7^1	k_{O2}
NF359	−942	−298	–	265
Nitrofurazone	−886	−242	−257 [a]	30
Nifurtimox	−930	−286	−260 [a]	166

[a] Values obtained from [34] and [35] by pulse radiolysis.

In this context, the biologically most important of these acceptors is obviously oxygen. In anaerobic conditions we must considerer the "futile" reduction of the nitro radical according to the following Equation 6

$$ArNO_2^{\bullet -} + O_2 \rightarrow ArNO_2 + O_2^{\bullet -} \quad (6)$$

From the obtained $E^1{}_7$ values the equilibrium constants for the electron transfer from nitro radical to oxygen (k_{O_2}) can be determined. Considering the above equilibrium (Eq. 6) and the Nernst equation a simple equation to calculate the k_{O_2} was described by Wardman [9]:

$$\log k_{O_2} = (-0.155 - E^1{}_7)/0.059 \quad (7)$$

The calculated k_{O_2} values starting from the peak potentials obtained from our CV measurements are listed in Table 1. From these values we can assume that the nitro radical anion from the compound NF359 is thermodynamically favored to "futile" reduction than NFZ and NFX. Consequently this possible new antichagasic compound shows a very good thermodynamic approach to produce redox cycling.

4. Conclusions

The above study is new confirmation about the extraordinary versatility of the voltammetric techniques wherein it is possible to study the behavior of one compound in different media in order to clarify its prototropic characteristics. According to this study we have found that the nitro radical anion from the new synthesized nitrofuran, NF359, decays by a disproportionation reaction in mixed medium, but in aprotic media decays by a dimerization reaction.

From the voltammetric measurements, we can conclude, that the nitro radical anion from NF 359 could be biologically formed via enzymatic reduction, because the energetic of formation did not differ much with that obtained for the well-know enzymatic reducible drugs as nitrofurazone and nifurtimox. On the other hand the lower half-life of its radical could represent an advantage of NF 359 with respect to the others drugs due to the minor permanence in the host, then reaching lower toxicity.

Beyond the specific case of the electrochemical characterization of some nitrofurans and the study of its nitro radical anion formation, this article illustrates the powerful role that electrochemistry can play in better understanding of biologically redox process, such us the redox activation of drugs in the treatment of infectious diseases. Finally, the adequate correlation between the $E^1{}_7$ values obtained by both cyclic voltammetry and pulse radiolysis implies that voltammetric measurements can be adequately used to obtain the $E^1{}_7$ parameter for these types of drugs, thus making the determination easier and cheaper than pulse radiolysis experiments.

5. Acknowledgements

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