Nitrofurazone and its Nitroheterocyclic Analogues: a Study of the Electrochemical Behavior in Aqueous Medium

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The biological action of drugs with potential antichagasic activities can be better understood by knowing their reduction mechanism. For this proposal, the electrochemical reduction of nitrofurazone (NF) and its analogues, nitrofurfurilidene thiosemicarbazone (NFS), nitrothiophene semicarbazone (NT), nitrotiothiophene thiosemicarbazone (NTS), was studied by differential pulse and cyclic voltammetries and chronoamperometry in aqueous medium by using a glassy carbon electrode. Those three synthetic analogues have oxygen and sulfur atoms arranged between the nitroheterocyclic ring (furan and thiophene, respectively) and the carbonyl group (semicarbazone and thiosemicarbazone, respectively). The respective hydroxylamine derivatives are the main products formed in acidic medium, being its formation linearly pH dependent. Although chronoamperometric data show that this reduction involves 4 electrons, its corresponding reduction voltammetric wave, in alkaline medium, is unfolded and a reversible reduction peak at a more positive potential appears. This peak is characterized as a nitro anion radical formation, whose decay is caused by a disproportionation reaction. Its kinetic stability was studied by the current ratio values of the R–NO₂/R–NO₂• redox couple and the second-order constant (k₂) was determined, being NTS the analogue that registered the highest stability.

Keywords: nitrofurazone, nitrofurazone analogues, nitro anion radical, aqueous medium, Chagas’ disease

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Introduction

Chagas’ disease, a parasitic illness caused by a kinetoplastid, Trypanosoma cruzi, is extremely neglected, representing a serious public health problem due to its high morbidity and mortality. According to the World Health Organization, around 10 million people are likely to be infected and nearly 10,000 deaths are estimated in Latin America per year. Unfortunately, only two drugs, benznidazole and nifurtimox, are currently available for chemotherapy, being effective only in the acute phase of the disease. Nifurtimox is a nitrofuran with an action mechanism that involves the nitro anion radical production, which in the oxygen presence impedes the T. cruzi capacity to detoxify the free radicals acting on it. On the other hand, benznidazole acts by binding its metabolites to nuclear DNA of the parasite and to its lipids and proteins. Despite the severe scenario, few new compounds have been assayed clinically. However, recent reviews highlight that new drug candidates have shown positive results in vitro and in vivo assays. Moreover, novel pharmaceutical formulations have been approached, targeting the delivery of nifurtimox and benznidazole. In spite of these efforts, this is a research area clearly underfunded and the development of new antichagasic agents, with less toxicity and fewer side effects, remains urgently needed.

Nitrofurazone (NF, 5-nitro-2-furaldehyde semicarbazone), Figure 1, was synthesized during the 1940s, being the first 5-nitrofuran drug introduced in therapeutics. This drug has proved to be a potential antichagasic drug, despite its toxicity, since it destroys T. cruzi through inhibition of the trypanothione reductase, enzyme found in the parasite but not in the host. Moreover, it is classically known that the nitro group reduction by nonspecific nitroreductases is the main mechanism for oxidative stress caused by nitroheterocyclic compounds, which gives support for its biological activity. Previous works have also demonstrated that NF and its NFOH prodrug inhibit the cruzain, indicating the possible existence of other action mechanisms against Chagas’ disease. Additionally, recent studies with prodrugs have shown improvements in the chemical properties of nitrofurans promoting an increase in biological activity and a decrease in toxicity.

Based on above description, three NF analogues were synthesized (Figure 1), in which oxygen and sulfur atoms were arranged between the nitroheterocyclic ring (furan and thiophene) and the carbonyl group (semicarbazone and thiosemicarbazone). These alterations might affect the biological activity as shown by Aguirre et al. who described anti-T. cruzi compounds similar to those herein presented.

Figure 1. Chemical structure of nitrofurazone (NF, W = O; Y = O) and its analogues: NFS (5-nitro-2-furfurylidene thiosemicarbazone, W = O; Y = S); NT (5-nitro-2-thiophene semicarbazone, W = S; Y = O) and NTS (5-nitro-2-thiophene thiosemicarbazone, W = S; Y = S).

It is accepted that the nitro anion radical and hydroxylamine derivatives are the main species responsible for the cytotoxic action of nitroheterocyclic compounds. For this very reason, electrochemical studies can be relevant to comprehend the redox cycles involved in those biological processes. Therefore, the differences in the biological activities of those kinds of compounds could be explained by electrochemical studies. Actually, the classical work published by Rozenski and coauthors showed a relationship between antimicrobial activity of nitroheterocyclic compounds and their reduction potential values (E1/2). On the contrary, the voltammetric peak potential values obtained for several nitroheterocyclic compounds do not unequivocally indicate a relationship with their trypanocidal activities, indicating that other different physicochemical parameters can set the action of biologically active compounds. However, depending on the experimental strategy, the cyclic voltammetry is a useful technique to calculate biological parameters, such as E1/2 (indicative of the biological nitro anion radical formation) and KO2 (thermodynamic indicator of the oxygen redox cycling), and to provide accessible diagnostic criteria in preliminary screening towards selecting agents with adequate biological performances.

The electrochemical reduction of nitroheterocyclic compounds follows a complex and well-known mechanism. Theoretically, the nitro group can receive up to six electrons in the complete reduction to the amine derivatives. Under anaerobic conditions or low oxygen pressure, the reduction process is similar to that observed for nitrobenzene, producing nitroso, hydroxylamine and amine derivatives. Moreover, the intermediate reversible redox couple represented by the R–NO2/R–NO2– can also be observed by using cyclic voltammetry. In an aprotic medium, mixed solvents or alkaline-pH medium, the kinetic stability of the nitro anion radical can be favored, since low proton availability is guaranteed. Under such conditions, the electrochemical behavior of NF has been extensively studied by using working electrodes such as mercury, gold, glassy carbon, carbon fiber and boron-doped diamond. Additionally, some of these works have reported studies on the electrochemical behavior of nitroheterocyclic compounds mainly focusing the
generation and stabilization of the R–NO₂/R–NO₂⁻ redox couple, highlighting the determination of the decay rate constant of the free radical.

This paper seeks to address a thorough study on the electrochemical NF behavior and its analogues (NFS, NT, NTS) in aqueous medium using a glassy carbon electrode by applying cyclic and differential pulse voltammetries and chronoamperometry and to emphasize the electrochemical R–NO₂/R–NO₂⁻ redox couple kinetic stability. The reversibility and electrode mechanism reaction were evaluated by the current ratio values dependence in relation to scan rate, compound concentrations and pH. The second-order rate constant of the nitro anion radical decay and its half-life time were also determined for all analogues studied. This work follows a methodology established with NFOH36 aiming to contribute to fully understand the reduction mechanism in aqueous media of this kind of compounds.

Experimental

Synthesis

The NF analogues (NFS, NT and NTS) were obtained from the reactions between the 5-nitrofurfural with the thiosemicarbazide, and also between 5-nitro-2-thienoic aldehyde with both carbazides, the semicarbazide or the thiosemicarbazide. All reactions were carried out in the proportion of 1:1, during 2 hours. One mmol of the respective aldehyde was solubilized in ethanol/water by using a 100 mL flask and kept under magnetic stirring. After solubilization, the carbazone was added slowly under mild heating until the precipitate is formed. Subsequently, the heating was ceased and the reaction was kept under stirring for 2 hours. Finally, the obtained product was filtered under reduced pressure and washed with water. The product was stored in a desiccator with silica gel and phosphorus pentoxide. The yields were: 80.7% for NFS, 87.5% for NT and 92.3% for NTS. The melting temperatures were determined in an automatic fusion capillary apparatus, Büchi M-565 model, being the melting point results obtained for NFS: 204-206 °C; NT: 223-224 °C; NTS: 231-233 °C.

Reagents and solutions

The stock solutions (0.05 mol L⁻¹) of NF (Avocado Company) and analogues (NFS, NT and NTS) were prepared through direct dissolution in ultrapure water and dimethylformamide (DMF, 1:1) assisted by an ultrasonic bath. The electrochemical experiments (cyclic and differential pulse voltammetries and chronoamperometry) were performed from the stock solution dilutions until final concentrations of 0.5 mmol L⁻¹, 0.1 mmol L⁻¹ and 0.5 mmol L⁻¹, respectively. The pH study of the final drug solution was accomplished with Britton-Robison Universal Buffer. All solutions were prepared by using analytical-grade reagents from Merck and ultrapure water from a Gehaka UV system.

Electrochemical apparatus

The cyclic voltammograms were recorded using an Autolab PGSTAT 30 potentiostat/galvanostat from Eco-Chimie, Utrecht, Netherlands, coupled to a 20 mL cell with a three-electrode system, being glassy carbon as the working electrode (GCE) and the other two, Ag/AgCl as the reference and Pt as auxiliary. The acquisition and treatment of data were performed using the GPES 4.9 program (Eco-Chimie). Dissolved air was removed from the solutions by 10 min bubbling with nitrogen. The pH control was measured with a Metrohm 654-pH-meter and the combined glass electrode at room temperature.

The GCE (Ø = 2 mm, Analion, Brazil) was manually polished with 1 µm diamond suspension in spray on Supra metallographic velvet (Arotec S/A, granulometry 1/4 µm). The GCE was rinsed with ultrapure water after having been polished. The GCE area (0.037 ± 0.004 cm²) was determined following methodology reported elsewhere and from cyclic voltammetric data obtained for K₃[Fe(CN)]₆.3H₂O and its diffusion coefficient (7.76 × 10⁻⁶ cm² s⁻¹) in KCl 0.5 mol L⁻¹ solution.

Prediction of diffusion coefficients

The diffusion coefficient predictions of the studied compounds in the aqueous phase with infinite dilution were carried out based on a previously published method, in which the Wilke-Chang equation was applied:

\[ D = \frac{7.4 \times 10^{-8}(xM)^{0.5}T}{\eta V^{0.6}} \]

where \( D \) is the diffusion coefficient of the solute in water (cm² s⁻¹), \( \eta \) is the viscosity of water (centipoise) at the temperature of interest (\( \eta = 0.8937 \) at 25 °C), \( M \) is the molar mass of water (g mol⁻¹), \( T \) is the temperature (K), \( x \) is the association parameter of water (2.53), \( V \) is the molar volume of the solute (cm³ mol⁻¹). The solute molar volume was calculated starting from the ratio between Van der Waals molecular volume and the Le Bas molar volume. The molecular volumes were calculated by a molecular
modeling study using the AM1 semiempirical method through the online Molinspiration internet free software.\textsuperscript{31}

**Determination of the number of electrons**

The number of electrons was determined at pH 4.02 with 0.5 mmol L\textsuperscript{–1} drug concentration by chronocamperometry with three levels of potential pulses. Level 1: potential 0 V, duration 0.2 s, sampling time 0.05 s. Level 2: potential –0.7 V, duration 15 s, sampling time 0.05 s. Level 3: potential 0 V, duration 0.2 s, sampling time 0.05 s. From the registered chronocamperograms, the linear relation between \(I_p\) as a function of \(t^{1/2}\) was plotted based on Cotrell equation.\textsuperscript{32,33}

**Determination of the rate constants**

Using the theoretical model developed by Olmstead-Nicholson,\textsuperscript{34,35} a working curve with the fit equation \(y = 0.0116 x^2 – 0.1239 x + 0.995\) (\(R = 0.999\)) was plotted, in which \(y = \omega\) and \(x = I_{pa}/I_{pc}\). The sphericity of the working electrode was considered zero (\(\rho = 0.0,\) planar electrode) for the condition \(a\tau = 4\). The interpolation of the ratio current values (\(I_{pa}/I_{pc}\)).\textsuperscript{32,33,35} obtained from experimental voltamograms, in the working curve leads to the \(\omega\) parameter determination, which incorporates the effects of the rate constant, drug concentration, and scan rate. The plot between \(\omega\) vs. \(\tau\) resulted in a linear fit relation described by the equation \(\omega = k_2 C\tau\), in which \(k_2\) is the rate constant value for the second-order reaction for the nitro anion radical decay obtained from this plot slope, since \(C\) is the drug concentration and \(\tau = (E_{1/2} – E_{c})/\nu\). The nitro anion radical stability was calculated by the half-life equation (\(t_{1/2} = 1/k_2 [R–NO\textsubscript{2}^\ast]\)), assuming that \([R–NO\textsubscript{2}^\ast]\) = drug concentration.

**Results and Discussion**

**Voltammetric reduction of nitrofurazone and its analogues**

NF and its analogues can be electrochemically reduced at the polished GCE in aqueous media, but their reductions demonstrate clear dependence on the pH changes. The recorded cyclic voltammograms in acidic medium (pH 4.02) showed only a single irreversible reduction wave (\(E_{pc1}\)) in the potential range that was studied. This behavior can be verified by the voltammograms shown in Figure 2. Previous works\textsuperscript{24,26,36} had shown that a second reduction wave could be observed for NF at more negative potentials than \(E_{pc1}\) values. From the peak potential values (–0.442 V NF, –0.430 V NFS, –0.410 V NT and –0.427 V NTS), it can be observed that the NFS and NTS had similar slight reduction facility in relation to the other analogues. Moreover, for slower scan rates these potential differences were less significant (Table 1). According to these facts, we could assume that all compounds reported have the same reduction mechanism and which the molecular modifications carried out on NF did not produce alterations on the voltammetric behavior of the analogues synthesized.

At pH 7.41, a similar voltammetric behavior was observed only at a slower scan rate. Differently from this, the main reduction peak can be unfolded, as it will be discussed in the next section. Table 1 shows the results confirming the similarity among the voltammetric behavior of these compounds. The current values recorded by CV were closely related, indicating that the reduction mechanism for all drugs involves the same number of electrons. Clearly, this assessment is possible only because the compounds underway present the same functional group and similar diffusion coefficients.\textsuperscript{33} Additionally, similar experiments were carried out by using differential pulse voltammetry (DPV), being the peak width at half height defined as \(W_{1/2} = 3.52 RT/\nu F\). The proximity of the \(W_{1/2}\) values confirmed that all compounds have the same reduction mechanism. It is also worth mentioning that differential pulse methodology can better discriminate effects kept constant before and after the pulse application. Consequently, this is an advantage for the electrochemical study of organic compounds using solid electrodes, since they frequently lead to electrode adsorption.\textsuperscript{32}

Therefore, the voltammetric wave that corresponds to the \(E_{pc1}\) value might follow the classic reduction mechanism for the nitro group in acidic medium,\textsuperscript{8,12,15,27,35} in which the hydroxylamine derivative is the main product formed as depicted below:

\[
\text{R–NO}_2 + 4e^- + 4H^+ \rightarrow \text{R–NOH} + H_2O
\]

Additionally, the \(I_{pc1}\) values varied linearly with \(\nu^{1/2}\). It is necessary to highlight that it was only possible to register this behavior by polishing the electrode surface between the current measurements as a function of scan rate, since the current decreased by about 30 percent after the first scan. In these conditions this charge transfer process is controlled by diffusion. Moreover, the reduction peaks were shifted towards more negative potential values with increasing scan rate, which clearly confirm an irreversible process of charge transfer. This behavior follows similar results previously registered.\textsuperscript{21,26,36}

From Figure 2, it is also possible to observe an oxidation peak (\(E_{pa1}\)) in the potential positive region, being the \(E_{pa1}\) values 0.314 V, 0.351 V, 0.326 V, 0.452 V for NF,
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Based on the above results, we assume that the hydroxylamine oxidation to the nitroso derivative corresponds to this peak. After performing run consecutively, the nitroso-hydroxylamine couple was not detected. Similarly, as already reported, the correspondent reversible wave is not registered at low scan rate values, being observed only the reaction as follows:

\[
\text{R–NHOH} \rightarrow \text{R–NO} + 2e^- + 2H^+
\]

The NTS analogue also showed unusual behavior, registering a second peak in the oxidation region, \(E_{p2} = 0.032\) V. It is noteworthy that the literature does not present similar record for the nitroheterocyclic compounds. The experimental registration showed that \(E_{p2}\) does not depend on main peak reduction, leading to the hypothesis that the molecule could have been adsorbed on the GCE surface. The presence of two sulfur atoms in the analogue, thionyl group and thiophene nucleus, might have been responsible for this phenomenon.

### Estimate of the electrons number in acidic medium

To better comprehend the voltammetric reduction mechanism of NF and its analogues, the number of electrons involved was estimated at pH 4.02 by using chronoamperometry. Table 2 shows these results from the electrolytic reduction of each analogue. The relationship between I as a function of \(t^{-1/2}\) showed excellent linear correlation, enabling the calculation of the number of electrons by applying the Cotrell equation.

Concerning the prediction of the diffusion coefficient, the Wilke-Chang model was applied considering that the dilutions of the stock solutions resulted in final solutions containing 0.5% maximum of DMF, being predominantly aqueous medium. Furthermore, the diffusion coefficient calculated for NF of \(7.76 \times 10^{-6}\) cm\(^2\) s\(^{-1}\) is slightly higher than its value experimentally determined at pH 4.0 of \(5.32 \times 10^{-6}\) cm\(^2\) s\(^{-1}\). Thus, from the results on Table 2, it is reasonable to affirm that practically 4 electrons are involved in the electrochemical reduction of the nitroheterocyclic compounds in acidic medium, confirming that the

### Table 1. Voltammetric results for reduction of NF and its analogues at pH 4.02 and 7.41 using GCE

<table>
<thead>
<tr>
<th>Drug</th>
<th>pH 4.02</th>
<th>pH 7.41</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV(^a)</td>
<td>DPV(^b)</td>
</tr>
<tr>
<td></td>
<td>(-E_{c,p1}) / V</td>
<td>(-I_{c,p1}) / µA</td>
</tr>
<tr>
<td>NF</td>
<td>0.359</td>
<td>8.89</td>
</tr>
<tr>
<td>NFS</td>
<td>0.364</td>
<td>7.88</td>
</tr>
<tr>
<td>NT</td>
<td>0.360</td>
<td>8.63</td>
</tr>
<tr>
<td>NTS</td>
<td>0.369</td>
<td>7.82</td>
</tr>
</tbody>
</table>

\(E_{initial} = 0.0\) V; \(E_{final} = –0.7\) V; \([\text{compound}] = 0.1\) mmol L\(^{-1}\); \(W_{1/2}\) = width of the peak at half height.

### Table 2. Numbers of electrons (\(n\)) obtained by using chronoamperometry at pH 4.02 regarding reduction 0.5 mmol L\(^{-1}\) of NF and its analogues

<table>
<thead>
<tr>
<th>Drug</th>
<th>(V / \lambda^1)</th>
<th>(D \times 10^6 / (\text{cm}^2 \text{s}^{-1}))</th>
<th>(\kappa)</th>
<th>Cotrell slope(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td>154.88</td>
<td>7.76(^e)</td>
<td>3.4 ± 0.2</td>
<td>7.1 ± 0.2 (\times 10^6)</td>
</tr>
<tr>
<td>NFS</td>
<td>163.75</td>
<td>7.50(^e)</td>
<td>3.6 ± 0.2</td>
<td>7.0 ± 0.2 (\times 10^6)</td>
</tr>
<tr>
<td>NT</td>
<td>164.02</td>
<td>7.50(^e)</td>
<td>3.4 ± 0.2</td>
<td>8.4 ± 0.1 (\times 10^6)</td>
</tr>
<tr>
<td>NTS</td>
<td>172.90</td>
<td>7.26(^e)</td>
<td>3.8 ± 0.2</td>
<td>8.2 ± 0.1 (\times 10^6)</td>
</tr>
</tbody>
</table>

\(^a\)Diffusion coefficients calculated by using Wilke-Chang equation;\(^b\)diffusion coefficient experimentally determined at pH 4.0;\(^d\)average values from 5 measurements;\(^e\)average values from 5 measurements, being \(R^2 = 0.999\).
hydroxylamine derivative is the main product formed as already discussed above.

**Effect of pH on the voltammetric reduction of Nitrofurazone and its analogues**

According to the results on Table 1 the nitroheterocyclic compounds’ reduction depends on the solution pH. From Figure 3, it can be observed that the main reduction wave registered in acidic medium suffers an unfolding at pH 7.41, and a shoulder appears around –0.5 V ($E_{cp2R}$) having a correspondent anodic peak, $E_{ap3}$.

The $E_{cp1}$ values displaced towards a more negative potential indicate the existence of a protonation equilibrium before the charge transfer process and that the decreased acidity complicates the electrode reaction. Figure 4 shows the correspondent pH-dependence plot for each drug, being the respective $\Delta E_{cp1}/\Delta pH$ ratios: 33 mV/pH, 36 mV/pH and 40 mV/pH for NF, NFS, NT and NTS, respectively, correspondent to the linear range in acidic medium. The slope values indicate that one proton is involved in the rate-determining step of the reaction in the pH range studied. Probably, this result is due to the occurrence of a fast protonation step preceding charge transfer. The $H^+$ ion, involved in the rate determining reduction step, corresponds to a second slow protonation reaction of the nitro group, which is further reduced to the nitroso intermediate. This reasoning has also been applied to the electrochemical behavior of nitrofurazone.21,24

Moreover, the investigation performed in the range $2 < pH < 12$ shows that a distinct process occurs on the working electrode at pH values higher than 7.0. These results are compatible with those already recorded for NF24 and NFOH.36

Zuman and collaborators19 described a detailed reduction mechanism of nitroheterocyclic compounds in acidic medium, which presents a set of reactions involving protonation and charge transfer steps. This sequence is defined as: $H^+$, $e^-$, $H^+$, $e^-$, 2$e^-$, 2$H^+$ totaling 4 protons and 4 electrons until the hydroxylamine derivative formation. Therefore, the series of equations described below corresponds to the linear range between $2 < pH < 7$ (Figure 4) and that represent the reduction complete mechanism of the nitro group ($E_{cp1}$, Figure 2):

$$
\text{R–NO}_2 + H^+ \rightarrow \text{R–NO}_2H^+
$$
$$
\text{R–NO}_2H^+ + e^- \rightarrow \text{R–NO}_2H^+
$$
$$
\text{R–NO}_2H^+ + H^+ \rightarrow \text{R–NO}_2H_2^{**}
$$
$$
\text{R–NO}_2H_2^{**} + e^- \rightarrow \text{R–N(OH)}_2 (=\text{R–NO} + \text{H}_2\text{O})
$$
$$
\text{R–NO} + 2e^- + 2H^+ \rightarrow \text{R–NHOH}
$$

From pH > 7.0 the $E_{cp1}$ values and the new peak, $E_{cp2R}$, do not change with pH. Besides the previous works,24,26,36 several reports in literature22,38-41 describe the possibility of the nitro anion radical formation in aqueous alkaline media. According to Wardman42 the anion radical is involved in the dissociation equilibrium, as follows:

$$
\text{R–NO}_2H^+ \leftrightharpoons \text{R–NO}_2^{**} + H^+
$$

and which the deprotonated form of the nitro anion radical is stable at physiological pH, since the pKa for this dissociation is usually < 7. For 5-nitrofurans values were
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1970 registered between $1 < \text{pKa} < 1.2$ and for nitroimidazoles this pKa value is around 6.42 Additionally, the pKa of the protonated anion radical, for most aromatic nitro compounds, is between 2 and 4.43 It can be observed that the potential difference between $E_{c, p1}$ and $E_{c, p2R}$ becomes larger as the proton availability decreases. This behavior corroborates the proposal that the three-electron reduction step to the hydroxylamine most probably involves first the protonation of the anion radical, which is followed by further reduction of the radical.39 Therefore, it is possible to assume that, at pH > 7.0, the $E_{c, p2R}$ corresponds to the one-electron reduction to the nitro anion radical and the peak which follows ($E_{c, p1}$) refers to the hydroxylamine derivative formation, as depicted below:

$$\text{R–NO}_2^- + e^- \rightleftharpoons \text{R–NO}_2^{•-}$$
$$\text{R–NO}_2^{•-} + 3e^- + 4H^+ \rightarrow \text{RNHOH}.$$  

In addition, $E_{a, p1}$ values are also pH dependent on the complete range studied. The linear relation slopes (53 mV/pH NF; 56 mV/pH NFS; 52 mV/pH NT and 46 mV/pH NTS) indicate the involvement of the same number of electrons and protons in the oxidation process of hydroxylamine to the nitroso derivative, as already registered for NF.24,26

Voltammetric generation and stabilization of nitro anion radical

Complementing the data herein reported, the electrochemical reduction of nitroheterocyclic can be evaluated under several criteria employed to demonstrate the generation and stabilization of the $\text{R–NO}_2^-/ \text{R–NO}_2^{•-}$ couple in aqueous media. Figure 5 shows the isolated register of the couple related to the nitro anion radical correspondent to the cyclic voltammograms of NFS recorded at pH 10.0. The increase of the scan rate displaced neither the reduction peak, $E_{c, p2R}$, nor its corresponding oxidation peak ($E_{a, p2R}$) significantly. This behavior indicates the reversibility of the system and that the increase of the scan rate facilitates the anodic current record. These results follow the same behavior registered for similar nitroheterocyclic compounds22,38-41 even when the drug is adsorbed on the work electrode, as registered for megazol.43

The free radical kinetic stability was evaluated through the current ratio ($I_{a, p3R}/I_{c, p2R}$) analysis corresponding to the one-electron reversible couple due to the $\text{R–NO}_2^-/\text{R–NO}_2^{•-}$ redox system. The current ratio values were obtained by simple procedure described by Nicholson,32,33 which included the following correction for the switching potential on the baseline. Due to the current overlap of the first peak with that of the second one, the value of $I_{c, p2R}$ corresponds to about 97% of the real value, taking into account the switching potential currents ($I_{\lambda}$). All current values refer to the absolute values measured from the zero current axis.

Both dissociation pKa and solution pH generally define the rate of the decay reaction of the free radical.42 Figure 6 shows that this kinetic stability increases with the pH, demonstrating a similar profile for all compounds in which $I_{a, p3R}/I_{c, p2R}$ ratio reaches a constancy, close to 1 at higher pH values. However, a slight decrease of $I_{c, p2R}$ values was observed (data not shown) increasing pH, which can indicate the occurrence of a protonation step preceding the charge transfer, suggesting the stabilization of the $\text{R–NO}_2^-/\text{R–NO}_2^H$ couple instead the deprotonated nitro anion radical at the lowest pH values.

Figures 7 and 8 show, respectively, the effects of the scan rate and drug concentration on $I_{a, p3R}/I_{c, p2R}$ ratio. The

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**Figure 5.** Cyclic voltammograms recorded at pH 10.01. [NFS] = 0.5 mmol L$^{-1}$. (A) 0.02 V s$^{-1}$; (B) 0.05 V s$^{-1}$; (C) 0.1 V s$^{-1}$; (D) 1.0 V s$^{-1}$.

**Figure 6.** Effect of pH values on $I_{a, p3R}/I_{c, p2R}$. (■) NF; (○) NFS; (▲) NT; (▽) NTS. [Compound] = 0.50 mmol L$^{-1}$. 

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former shows that the scan rate had more influence when the voltammograms were registered at the lower values of pH, mainly for NF, while the latter shows that the current ratio decreases as the drugs concentration increases. Additionally, these results confirm the important role of the pH, since at pH > 9 the current ratio values are around 1. All analogues present the same behavior.

These diagnostic criteria satisfies the homogeneous process coupled to the electrode, which has an irreversible chemical reaction of a later stage reversible charge transfer. In fact, the literature results show that the nitro anion radical decay by a disproportionation reaction in protic media and by dimerization in aprotic medium. Therefore, the behavior described herein leads to a conclusion that NF and its analogues undergo an irreversible chemical reaction of disproportionation after the nitro anion radical generation (E_C1 mechanism). Moreover, this case can be distinguished from the first-order kinetics by the dependence of the electrochemical response (the current ratio) on the reagent concentration. The overall mechanism is described below.

$$2\text{R–NO}_2^* + 2\text{H}^+ \rightarrow \text{R–NO}_2^+ + \text{R–NO} + \text{H}_2\text{O}$$

Figure 9 shows the plots where a linear relationship between the kinetic parameters ($\theta$ vs. $\tau$) is obtained at different pH values while Table 3 presents the values of $k_2$ and the half-time life ($t_{1/2}$) for all the analogues. Similarly to the previous work and compared with literature results for other nitroheterocyclic compounds in aprotic medium, the $k_2$ values herein registered present at least 1 order of magnitude larger than those registered in an aprotic or mixed medium, indicating that nitro anion radical is less stable in protic medium.

As there are no indications that the $\text{R–NO}_2^*$ itself reacts in water ($k = 0$), the protic medium can favor the nitro anion radical decay providing enough protons for the $\text{R–NO}_2\cdot\text{H}^+$ formation, facilitating the fast protonation reaction between the conjugate base $\text{R–NO}_2^–$ and the neutral free radical formed, as depicted below:

$$\text{R–NO}_2\cdot\text{H}^+ + \text{R–NO}_2^* (+ \text{H}^+) \rightarrow \text{R–NO}_2^+ + \text{R–NO} (+ \text{H}^+)$$

Based on discussion above, it is evident that the nitro anion radical stability is favored in alkaline medium. Among the compounds, NFS showed less variation for $k_2$ and $t_{1/2}$, NF and NT had close results, and NTS registered the highest stability. Nevertheless, there are several reports showing those phenomena on the electrode surface play a decisive role in the nitro anion radical stabilization.
Adsorptive processes can prevent the nitro radical anion surface protonation. Previous works have shown that the GCE polishing using diamond powder produced a similar effect to that observed in an aqueous medium after the alumina removal from the electrode surface by sonication in ethanol. The performed comparisons with mercury and gold electrodes reinforce this behavior. As also demonstrated, the nitro radical anion was not sufficiently and kinetically stable to produce a couple using a mercury electrode in protic medium, even at alkaline pH. Therefore, the possible existence of a nonspecific adsorption of NTS on electrode surface can be associate with its highest \( t_{1/2} \) value, since adsorptive processes on GCE might be preventing the nitro anion radical surface protonation. Furthermore, the treatment and activation of the CGE surface using by polishing with diamond powder can be contributing for a hydrophobic environment formation in the interface that favors the nitro anion radical stability in the diffusion layer.

**Conclusion**

NF and its analogues were reduced in acidic medium using a GCE, producing only one reduction peak involving four electrons due to the hydroxylamine derivative formation. Furthermore, the generation and stabilization of the nitro anion radical in alkaline medium were observed, showing that even in aqueous medium this behavior is possible as the protons low availability make the free radical protonation slower, which allows its detection on the time scale of the cyclic voltammetric technique. Moreover, this work confirms previous results showing that the activation of GCE surface through polishing with diamond powder contributed to the kinetic stability of the nitro radical anion due to the hydrophobic properties of the interface, hindering or even suppressing the radical anion protonation in the diffusion layer. The kinetics results found for the analogues showed that NTS presented the lowest values for \( k_2 \) and \( t_{1/2} \), while the highest half-time life, although these results are not enough to distinguish the significant differences due the molecular modifications carried out on NF.

### Table 3. Values of \( k_2 \) and \( t_{1/2} \) for the reaction of disproportionation of nitro anion radical in different pH values; [compound] = 0.5 mmol L\(^{-1}\)

<table>
<thead>
<tr>
<th>pH</th>
<th>( k_2 \times 10^2 ) (L mol(^{-1}) s(^{-1}))</th>
<th>( t_{1/2} ) s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF</td>
<td>NFS</td>
</tr>
<tr>
<td>8.03</td>
<td>16.74</td>
<td>20.90</td>
</tr>
<tr>
<td>9.02</td>
<td>11.43</td>
<td>15.84</td>
</tr>
<tr>
<td>10.01</td>
<td>7.07</td>
<td>13.29</td>
</tr>
<tr>
<td>12.05</td>
<td>5.68</td>
<td>13.28</td>
</tr>
</tbody>
</table>

**Supplementary Information**

Supplementary information is available free of charge at [http://jbcs.sbq.org.br](http://jbcs.sbq.org.br) as PDF file.

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**References**

42. Wardman, P.; Environ. Health Perspect. 1985, 64, 309.

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Nitrofurazone and its Nitroheterocyclic Analogues: a Study of the Electrochemical Behavior in Aqueous Medium

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Chemical characterizations of the synthesized analogues

The 1H NMR spectra were recorded in a Bruker Avance DPX-300 spectrometer, using DMSO-d6 as solvent and TMS as internal reference. NFS: 1H NMR (300 MHz, DMSO-d6) δ 7.63 (d, 1H, J 3.66 Hz, H-aromatic), 7.78 (d, 1H, J 3.66 Hz, H-aromatic), 7.96 (s, 1H, H-azometine), 8.02 (s, 1H, H-terminal amine), 11.83 (s, 1H, H-amine). NT:

1H NMR (300 MHz, DMSO-d6) δ 6.59 (s, 2H, H-amine), 7.43 (d, 1H, J 3.72 Hz, H-aromatic), 8.08 (d, 1H, J 3.72 Hz, H-aromatic), 8.03 (s, 1H, H-azometine), 10.76 (s, 1H, H-amine). NTS: 1H NMR (300 MHz, DMSO-d6) δ 7.53 (d, 1H, J 4.23 Hz, H-aromatic), 8.07 (d, 1H, J 4.23 Hz, H-aromatic), 8.04 (s, 1H, H-terminal amine), 8.18 (s, 1H, H-terminal amine), 8.45 (s, 1H, H-azometine), 11.82 (s, 1H, H-amine).

Figure S1. Cyclic voltammograms recorded at pH 10.01. [Compound] = 0.5 mmol L⁻¹; (A) 0.02 V s⁻¹; (B) 0.05 V s⁻¹; (C) 0.1 V s⁻¹; (D) 1.0 V s⁻¹.