

# Nitroradical Anion Formation from some Iodo-Substituted Nitroimidazoles

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## Abstract

The electrochemical behavior of iodo nitroimidazole derivatives such as 1-methyl-4-iodo-5-nitroimidazole (M-I-NIm) and 1-methyl-2,4-diiodo-5-nitroimidazole (M-I<sub>2</sub>-NIm) and the parent compound 1-methyl-5-nitroimidazole (M-NIm), was studied in protic, mixed and non-aqueous media. The electrochemical study was carried out using Differential Pulse Polarography (DPP), Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) and coulometry and as working electrodes mercury and glassy carbon were used. As can be expected, in all media, the effect of introduce iodo as substituent in the nitroimidazole ring produced a decrease of the energy requirements of the nitro group reduction. Certainly, this fact can be explained by the electron withdrawing character of the iodo substituent that acts diminishing the electronic density on the nitro group thus facilitating their reduction. In all the studied media the reduction of M-NIm produced a detectable signal for a nitro radical anion derivative. In the case of M-I-NIm the nitro radical anion was only detectable in both mixed and non-aqueous media. On the other hand the nitro radical anion for the M-I<sub>2</sub>-NIm was detected only in non-aqueous medium. When glassy carbon electrode was used as the working electrode in a mixed medium a detectable nitro radical anion derivative appeared for all compounds, thus permitting an adequate comparison between them. The obtained values of  $k_2$  for M-NIm, M-I-NIm and M-I<sub>2</sub>-NIm in non-aqueous medium were  $5.81 \times 10^2$ ,  $132 \times 10^2$  and  $1100 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$ , respectively. From the obtained  $k_2$  and  $t_{1/2}$  values in this medium, it is concluded that there is a direct dependence between the presence of iodo substitution in the nitroimidazole ring with the stability of the nitro radical anion.

**Keywords:** Nitro radical anion, Nitroimidazoles, Reduction, Decay constants

## 1. Introduction

The investigation of different types of nitrocompounds has been a very active research topic during several years probably due to its many applications. There are a lot of nitrocompounds which possess interesting biological properties such as antimicrobial [1, 2], antituberculosis [3], anti-protozoa and anticancer [4, 5] activities. Increasingly, the treatment of protozoa infections, such as Chagas disease, has become an important problem due to the emergence of drug resistance. Consequently, a series of nitro derivatives has been synthesized with the aim of finding new drugs. In previous studies, a 5-nitroimidazole derivative, megalol, showed a good activity against *Trypanosoma cruzi*, the causative agent of Chagas disease, better than the classic antichagasic drugs nifurtimox and benznidazole [6]. In that work the results indicated that nifurtimox and related compounds act as redox cycles, whereas, the 5-nitroimidazole megalol essentially acts as thiol scavenger particularly for trypanothione, the cofactor for trypanothione reductase, an essential enzyme in the detoxification process. The mechanism of megalol would imply the nitro radical anion formation which is converted to the nitroso derivative as a consequence of a disproportionation reaction.

In view of the good results obtained with megalol, a renewal interest in to obtain new synthesized nitroimidazole

compounds required for pharmacological and toxicological investigations have been generated. In this way, a series of iodo nitroimidazole derivatives (Figure 1) were generated in the new synthetic route for obtaining megalol and are being studied for possible pharmacological applications.

In our laboratory we have conducted several electrochemical studies of nitro compounds in order to characterize the redox activation that implies the one electron transfer of the nitro group to produce the nitro radical anion [7, 8–11]. In such studies we characterized the reduction potential of the  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$  couple, a well recognized parameter as a very appropriate index to define the type of biological

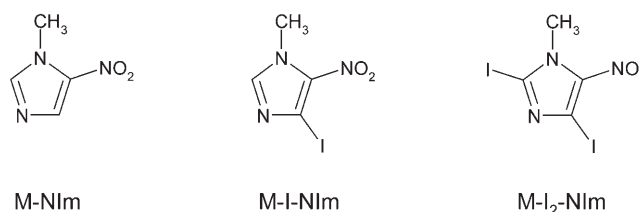


Fig. 1. Chemical structure of iodo nitroimidazole derivatives 1-methyl-4-iodo-5-nitroimidazole (M-I-NIm), 1-metil-2,4-diíode-5-nitroimidazole (M-I<sub>2</sub>-NIm) and the parent compound 1-methyl-5-nitroimidazole (M-NIm).

properties of the different nitrocompounds as was demonstrated by Olive [12] on a series of nitroheterocyclic compounds. Furthermore, in those previous studies was revealed the voltammetric feasibility of quantitatively characterize the formation and stabilization of the nitro radical anion.

In the present work we are interested in to study the electrochemical significance of adding one or two iodo atoms in the 1-methyl-5-nitroimidazole moiety. The study will be mainly focused to reveal the effect of these iodo substituents in the formation and stability of the nitro radical anion derivatives in different media.

## 2. Experimental

### 2.1. Reagents and Solutions

All the compounds were synthesized and characterized by one of us. 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 60/40:DMF/citrate buffer (0.015 M) + KCl (0.3 M) for mixed media were used. The pH was adjusted with little aliquots of concentrated NaOH or HCl, respectively. Dimethylformamide (DMF) and tetrabutyl ammonium perchlorate (TBAP) as solvent and supporting electrolyte were used in non-aqueous 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  $25.0 \pm 0.1$  °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<sup>2</sup> and a glassy carbon as working electrodes and a platinum wire as a counter electrode were used. All potentials were measured against Ag/AgCl 3 M.

For Differential Pulse Polarography (DPP) the CGME stand was used in a CGME mode, for cyclic voltammetric experiments the CGME stand was used as SMDE mode (static mercury drop electrode) and for DPV the GCME stand was used as C2 mode, i.e., for solid electrode operation, under the following conditions: scan rate 20 mV/s, pulse amplitude 50 mV, pulse width 50 mV, sample width 20 ms and pulse period 200 ms.

For the kinetic analysis, the return-to-forward peak current ratio  $I_{pa}/I_{pc}$  for the reversible one electron couple ( $\text{RNO}_2/\text{RNO}_2^{\cdot-}$ ) was measured for each individual cyclic voltammogram according to the procedure described by

Nicholson [13]. The scan rate was varied between 0.1 to 10 V/s.

Using the theoretical approach of Olmstead et al. for dimerization or disproportionation [14, 15], the  $I_{pa}/I_{pc}$  values measured experimentally at each scan rate were inserted into a working curve to determine the parameter  $\omega$ , which incorporates the effects of rate constant, drug concentration and scan rate. A plot of  $\omega$  versus  $\tau$  resulted in a linear relationship described by the equation

$$\omega = k_2 C_o \tau$$

Where  $k_2$  is the second-order rate constant for the decomposition of  $\text{RNO}_2^{\cdot-}$ ,  $C_o$  is the nitrocompound concentration and  $\tau = (E_\lambda - E_{1/2})/\nu$ , where  $E_\lambda$  is the switching potential,  $E_{1/2}$  is the half wave potential and  $\nu$  is the scan rate. Consequently we can obtain the second order rate constant for the decomposition of the nitro radical anion from the slope of the straight line  $\omega$  versus  $\tau$ . The assumption that the decomposition of  $\text{RNO}_2^{\cdot-}$  follows second-order kinetics is supported by the obtained linearity between the kinetic parameters  $\omega$  and  $\tau$ .

## 3. Results and Discussion

In order to investigate the influence of electron withdrawing substituents in the nitroimidazole moiety on the electrochemical reduction of the nitro group we have synthesized two iodo derivatives (Figure 1).

### 3.1. Electrochemical Behavior in Protic Medium

In protic medium (0.1 M Britton-Robinson buffer + 0.3 M KCl / EtOH:70/30) all compounds were reduced at dropping mercury electrode (DME), producing signals which were dependent of the number of iodo substituents in the molecule. Figure 2 shows the differential pulse polarograms (DPP) at four different pHs for each nitroimidazole derivative. A direct relation between the number of iodo and the complexity of the signals can be observed. In this way, M-NIm (Figure 2A) shows one reduction signal between pHs 2 and 7.5 and two signals above pH 8. This behaviour was previously observed [16] for the 4-nitroimidazole reduction in protic medium wherein the signal I corresponds to the 4-electron and 4-protons nitro reduction to form the hydroxylamine derivative. This mechanism was corroborated by coulometry where at pH 5 the calculated total transferred electrons were  $4.02 \pm 0.065$ . Consequently, the overall mechanism at acid and neutral media is described by:



When the pH increased a splitting of this signal is produced, being possible to obtain an isolated signal due to the one electron reduction of the nitro group, to produce the nitro

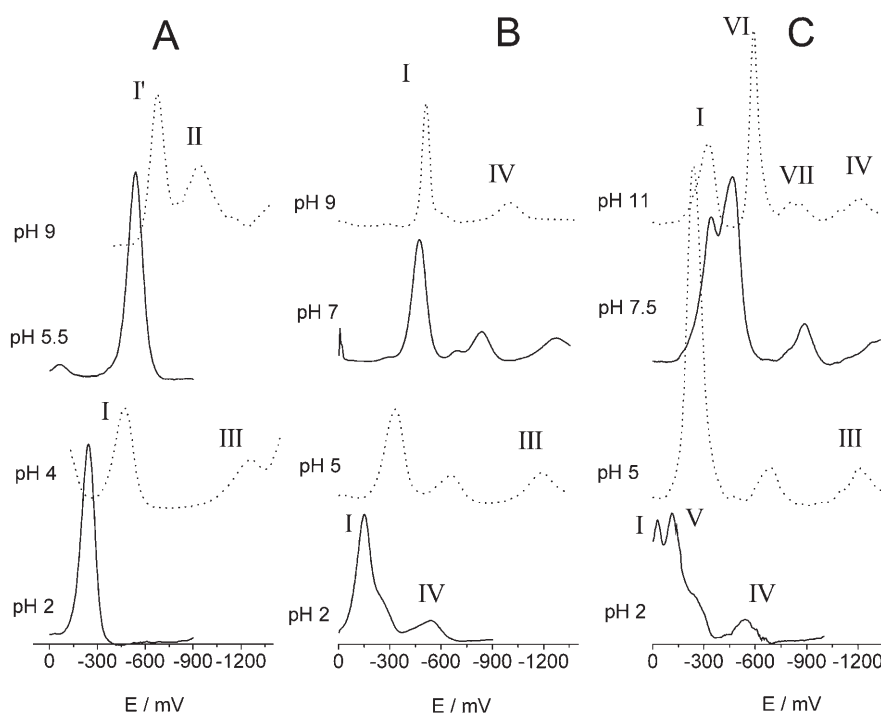


Fig. 2. Differential pulse polarograms of M-NIm (A), M-I-NIm (B) and M-I<sub>2</sub>-NIm (C) in ethanol/Britton-Robinson buffer medium at different pHs.

radical anion, which is further reduced to the hydroxylamine derivative (signals I' and II in Fig. 2A):



The above observed behavior was corroborated by cyclic voltammetry at the mercury electrode. At pH 5 (Fig. 3A) we can observe two reduction peaks, peak I due to the four electron reduction of the nitro group to the hydroxylamine derivative, and peak III, at sufficiently higher potentials, due to the C=N reduction of the imidazole ring. In the reverse scan a new peak IIa, due to the hydroxylamine oxidation, is observed according to the following equation:



Changing the pH to the basic zone (Fig. 3B) a splitting of peak I in the new peaks I'c and I'c is observed, also a new anodic peak I'a appear, which correspond to the nitro radical anion oxidation. In this Figure a short cyclic voltammogram is included (dotted line) showing the isolation of the redox couple  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$ . Furthermore, from this Figure we can observe a notorious increase of the oxidation current I'a, which is due to the nitro radical anion oxidation, when short and large scans are compared. This result agree with the fact that in the case of the short scan the radical remains much less time in the immediacy of the electrode before oxidizing in the back scan thus avoiding further reactions.

Selecting appropriately the switching potential is possible to work with the couple in isolation which is characterized by its current ratio values,  $I_{p,a}/I_{p,c}$ . For M-NIm in basic medium an EC mechanism was determined since the  $I_{p,a}/I_{p,c}$  value increases concomitant the sweep rate of the experiment was increased too (Figure 3C).

According to the literature information [17] the chemical reaction coupled to the electron transfer in the EC mechanism in a basic protic medium correspond to a disproportionation reaction (Eq. 5) which can be characterized by its corresponding rate constant,  $k_2$ :



According to the previously informed methodology [14] we have obtained the decay rate constant ( $k_2$ ) at different pH values. Figure 3D shows the correlation existing between  $k_2$  values and the pH of reaction medium, concluding that the nitro radical anion is more unstable whereas the pH is lower. Moreover, the dependence of the  $k_2$  value with pH obeys the following regression curve:  $\log k_2 = 6.36 - 0.27\text{pH}$  ( $r = 0.993$ ). By extrapolation from this relation the  $k_2$  value at different pHs was obtained. In this way and considering that the physiological aqueous pH is 7.4, the extrapolated rate constant was  $2.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ . Comparing this value to that previously obtained using the same procedure for 4-NIm,  $7.48 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$  [16] it is clear that the methyl substitution in position 1 of the imidazole ring produces a more stable nitro radical anion.

When iodo was incorporated in the M-NIm moiety producing both M-I-NIm and M-I<sub>2</sub>-NIm compounds, a

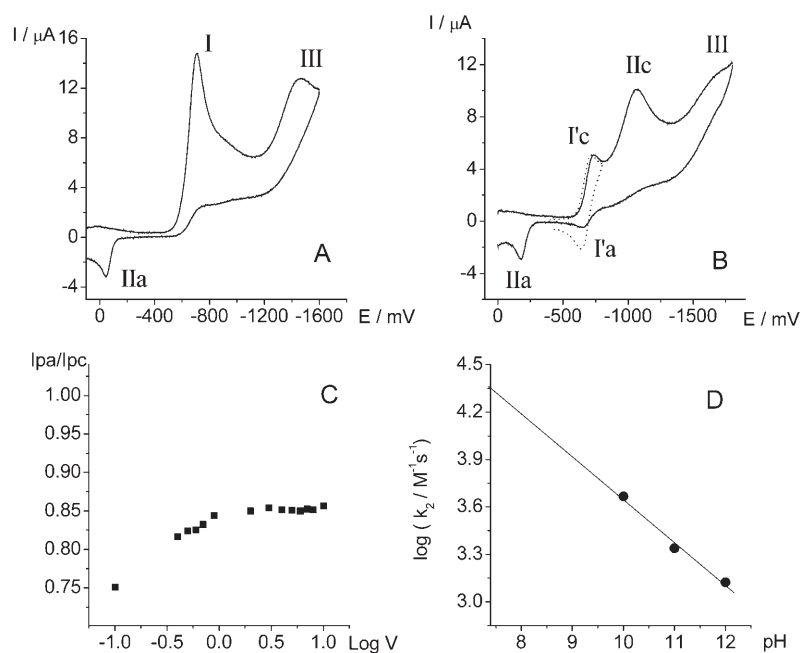


Fig. 3. Cyclic voltammograms on mercury electrode of 1 mM M-NiM in ethanol/Britton-Robinson buffer 0.1 M at pH 5 (A) and pH 10 (B). Sweep rate  $1 \text{ V s}^{-1}$ . Dashed line shows a short sweep with the  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$  isolated couple. C) Current ratio dependence on sweep rates of the  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$  couple from cyclic voltammograms of 1mM M-NiM in ethanol/Britton Robinson buffer at pH 10. D) pH dependence of the natural decay,  $k_2$ , of the nitro radical anion.

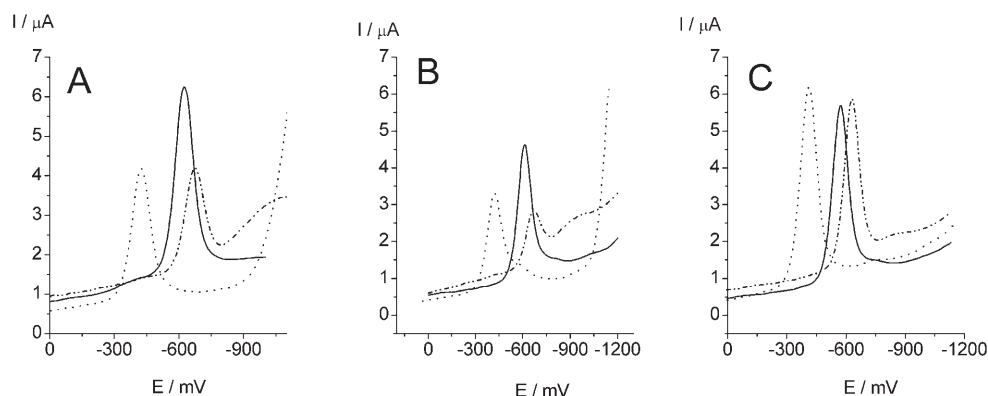


Fig. 4. Differential pulse voltammograms of M-NiM (A), M-I-NiM (B) and M-I<sub>2</sub>-NiM (C) in ethanol/Britton-Robinson buffer medium at different pHs obtained using glassy carbon electrode. (.....) pH 3; (—) pH 7 and (· · · · ·) pH 10.

completely different redox behavior was observed in comparison with M-NiM. The presence of iodo in the molecule produced not reliable polarograms with a series of minor peaks probably due to an interference between mercury and iodo (Fig. 2B, C).

With the aim of deepening in the redox mechanism, a series of cyclic voltammetric experiments were conducted. A very sharp peaks were obtained then we can conclude the adsorptive character of the nitro reduction peak (data not shown). Also, the slope of  $\log I_p$  vs.  $\log V$  plots, were very close to 1 for both compounds, corroborating the strong adsorption of the molecule over the mercury electrode.

Thinking that the adsorption phenomenon could be due to interactions between mercury and the iodo atoms, we change the electrode material by glassy carbon, to avoid this

interaction. Surprisingly the observed voltammogram was modified considerably when carbon was changed by mercury as the electrode material. Figure 4 shows the differential pulse voltammograms at pHs 3, 7 and 10, revealing only one signal, which is due to the 4-electrons and 4-protons nitro reduction. Consequently no distortions and new signals in the voltammograms for M-I-NiM and M-I<sub>2</sub>-NiM were observed. Consequently we can confirm the hypothesis that an interaction between mercury and iodo affects considerably the voltammetric response over mercury in aqueous protic medium. With the objective of quantify the effect of the iodo substitutions on the energetic of the nitro reduction process, we have calculated the corresponding peak potential values. The potential peak values for the nitro group reduction at glassy carbon electrode are shown

Table 1. Peak potentials values (mV), obtained from cyclic voltammetric experiments using glassy carbon electrodes at different pH in protic medium.

pH	M-NIm	M-I-NIm	M-I <sub>2</sub> -NIm
4	-432	-422	-412
8	-620	-612	-572
12	-672	-667	-630

Table 2. Peak potentials values (mV), obtained from cyclic voltammetric experiments using glassy carbon electrodes, for signal I<sub>c</sub> and I<sub>2c</sub> at different pH in mixed medium.

pH	M-NIm		M-I-NIm		M-I <sub>2</sub> -NIm	
	I <sub>c</sub>	I <sub>2c</sub>	I <sub>c</sub>	I <sub>2c</sub>	I <sub>c</sub>	I <sub>2c</sub>
4	751	-	-735	-	-646	-
8	-855	-1329	-836	-	-778	-
12	-839	-1377	-825	-1306	-782	-1189

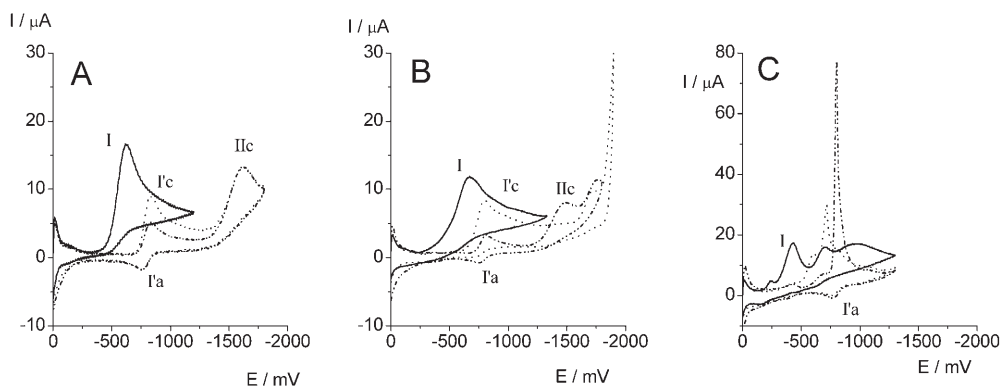


Fig. 5. Cyclic voltammograms of 1 mM of M-NIm (A), M-I-NIm (B) and M-I<sub>2</sub>-NIm (C) at pH 3, 7 and 11 in DMF/Citrate + KCl 0.3 M medium at Static mercury drop electrode. Sweep rate 1 V s<sup>-1</sup>.

in Table 1, being clear that the presence of iodo in the molecules produced a more easy reduction of the nitro group. Certainly, this fact can be explained by the electron withdrawing character of the iodo substitution that acts diminishing the electronic density on the nitro group thus facilitating their reduction.

The most important difference between both electrodes is that only in the case of the mercury electrode is possible to appreciate the splitting of the reduction signal due to the one-electron reduction of M-NIm to produce the corresponding nitro radical anion derivative.

### 3.2. Electrochemical Behavior in Mixed Medium

In mixed medium (60/40: DMF/Citrate buffer + 0.3 M KCl) the redox behavior of M-NIm, obtained by differential pulse polarography, was very close to that observed in protic medium. In the case of the iodo substituted compounds the observed adsorption interference in the mercury electrode at protic medium was substantially diminished in mixed medium. However exists an important difference between both reaction media, since in mixed medium, i.e., in the presence of a non-aqueous solvent as major component, the splitting of signal I was observed for the three compounds, indicating that the nitro radical anion is more stable in this medium.

By using cyclic voltammetry, the signals for M-NIm and M-I-NIm had a diffusion control, which permit us to identify clearly each electron transfer (Fig. 5A and 5B). At acid medium, the nitro reduction (signal I) occurs via 4-electron

and 4-protons according to Equation 1 but when pH was increased, the splitting of this signal in two new signals occurred i.e. a first reversible couple (I<sub>c</sub>, I<sub>a</sub>) and a second irreversible peak (I<sub>2c</sub>) (Eqs. 2 and 3). On the other hand, M-I<sub>2</sub>-NIm (Fig. 5C) shows a very sharp distortion due to an adsorption phenomenon complicating the cyclic voltammograms. However also is possible to observe some anodic signal due to the nitro radical anion formation (signal I<sub>a</sub>). By changing the working electrode from mercury to glassy carbon, a cyclic voltammetric response without adsorption complications was obtained for the three compounds (Figs. 6 A, B and C). In this case, the reduction process of the nitro group shows one signal between pH 2 and 8, and the splitting of this signal over this pH value. Comparing the peak potential values (Fig. 6D) obtained for each cathodic signal, it is clear that the nitro group of M-I<sub>2</sub>-NIm is easier to be reduced in all the pH range, and is still more clear the effect of iodo substitution on the nitro radical anion reduction potential (Signal I<sub>2c</sub>), where the quantity of iodo present in the molecule is proportional to the peak potential values. In Table 2 we can observe a similar behaviour that observed in protic medium, with lower values in the iodo substituted compounds, revealing the withdrawing character of the iodo substituent.

As the nitro radical anion signal was observed using both mercury and glassy carbon electrodes, a detailed study in order to determinate its stability and the effect of substituents in its stability was conducted in both media.

Figure 7 shows the cyclic voltammograms obtained for the redox couple RNO<sub>2</sub>/RNO<sub>2</sub><sup>•-</sup> using mercury electrode for M-NIm and M-I-NIm (Fig. 7A and B) and glassy carbon

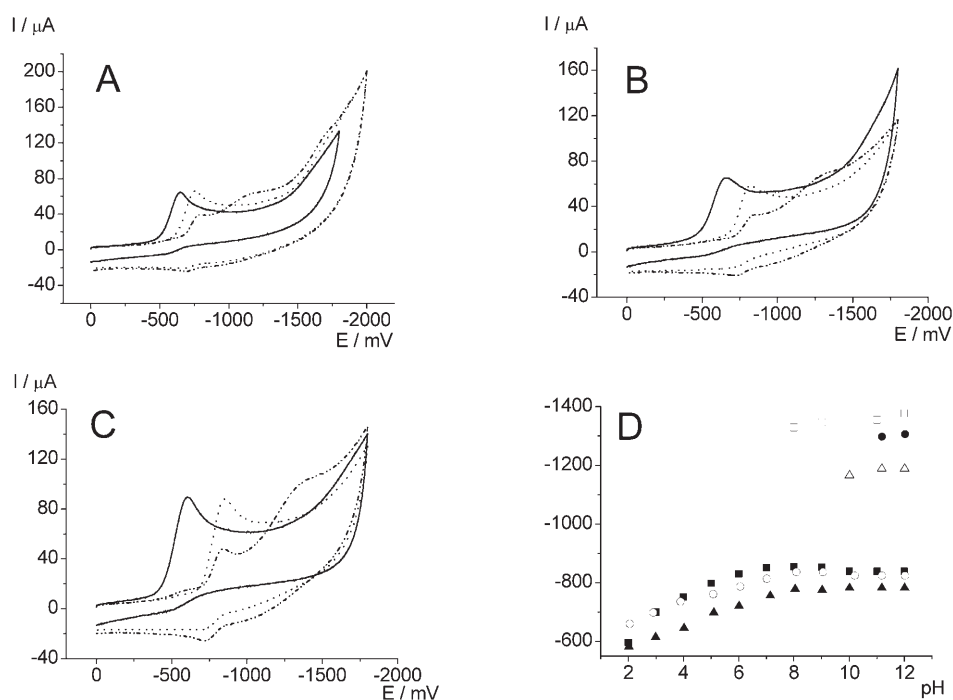


Fig. 6. Cyclic voltammograms of 1 mM of M-Nim (A), M-I-NIm (B) and M-I<sub>2</sub>-NIm (C) at pH 3, 7 and 11 in DMF/Citrate + KCl 0.3 M medium at glassy carbon electrode. Sweep rate 1 Vs<sup>-1</sup>. D) Peak potential dependence with pH for M-Nim (□), M-I-NIm (●) and M-I<sub>2</sub>-NIm (Δ) in DMF/Citrate + KCl 0.3 M medium.

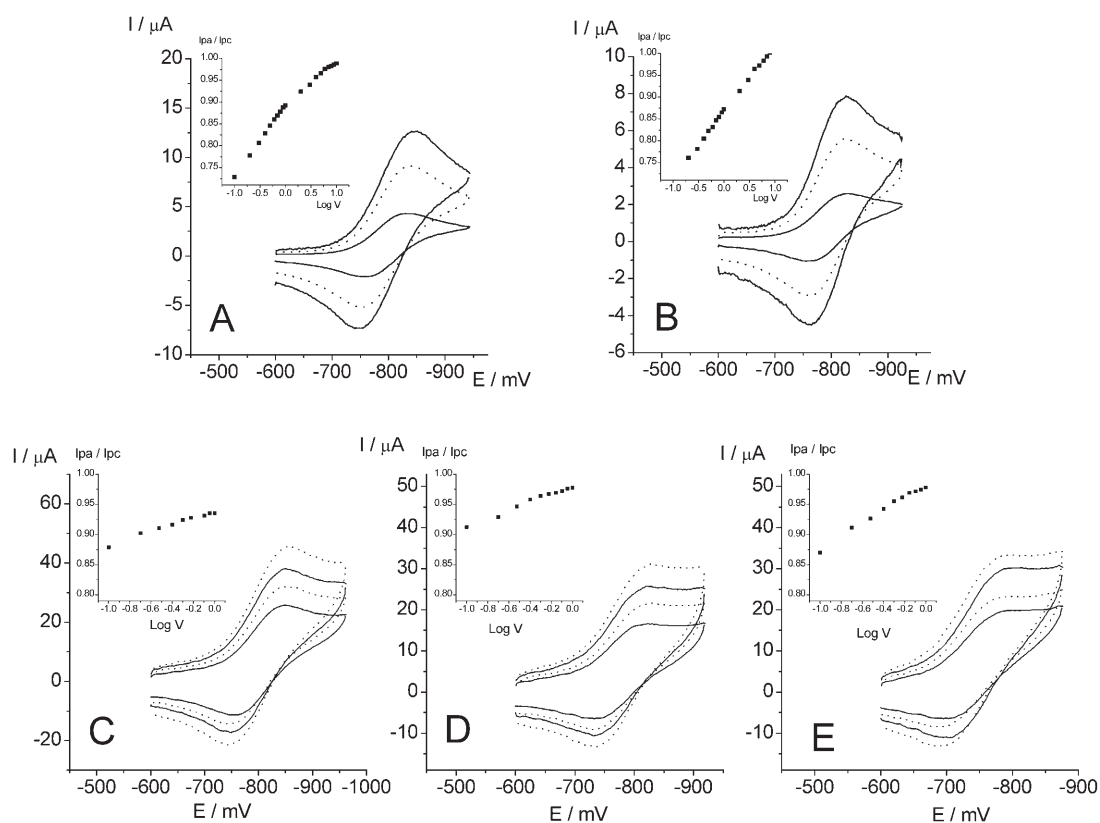


Fig. 7. Cyclic voltammograms showing the reversible couple due to the one-electron reduction of M-NIm (A), M-I-NIm (B) at mercury electrode, pH 10 and M-NIm (C), M-I-NIm (D) and M-I<sub>2</sub>-NIm (E) at glassy carbon electrode pH 12 in DMF/Citrate + KCl 0.3 M medium. Inset: Current ratio dependence on sweep rates of the RNO<sub>2</sub>/RNO<sub>2</sub><sup>-</sup> couple from cyclic voltammograms for each experiment.

Table 3. Decay constant ( $k_2 \times 10^{-2}$  [Ms] $^{-1}$ ) for the nitro radical anion electrochemically formed in mixed medium at different pHs and different electrodes.

	M-NIm	M-I-NIm	M-I <sub>2</sub> -NIm
<b>Mercury</b>			
pH 9	175	173	–
pH 10	41	64	–
<b>Glassy carbon</b>			
pH 10	10.4	12.4	10.9
pH 11	7.1	6.5	7.3
pH 12	3.3	3.5	6.6

electrode for all the compounds (Fig. 7C, D, and E), also the corresponding dependence of the current ratio ( $I_{p,a}/I_{p,c}$ ) with the sweep rate are shown (Inset Fig. 7). From these experiments and studying the effect of the nitro compound concentration on the current ratio (data not shown) we concluded that in this medium the nitro radical anion decays according to an *ECi* mechanism, wherein the chemical reaction corresponds to a disproportionation in the same way that in protic medium.

Consequently, in mixed medium, we have calculated the decay constant values,  $k_2$ , at different pHs and different electrodes, which are shown in Table 3. From these results we can conclude that the stability of nitro radical anion is not affected by the presence of iodo as substituent in the molecule since the constant values of the three compounds are quite similar when the pH remain constant using both mercury and glassy carbon electrodes. However the stability of the radical is strongly affected with pH changes showing an enhanced stability at alkaline pHs (Table 3).

Finally the electrode material seems to affect also the stability of the radical, although we have only one pH value to compare (pH 10), the decay constant values obtained with mercury are slightly higher than that obtained below the same conditions with glassy carbon electrode.

### 3.3. Electrochemical Behavior in Non-Aqueous Medium

With the aim of eliminate all adsorption interferences we have also studied a totally non-aqueous medium (100% DMF + TBAP 0.1 M). The stability of the nitro radical anion was studied by cyclic voltammetry using mercury electrode. On these conditions the couple  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$  was detected without any adsorption complications (Fig. 8) for the three compounds. This behavior was confirmed according to the slopes of the  $\log I_p$  vs.  $\log \nu$  plots, with values of 0.46, 0.41 and 0.44 for M-NIm, M-I-NIm and M-I<sub>2</sub>-NIm respectively.

For M-I<sub>2</sub>-NIm, in non-aqueous medium, the behavior was markedly different with the emergence of a prepeak before the nitro reduction couple (Fig. 8C). In a first instant this prepeak was evaluated as a consequence of an acid-base equilibrium, since this phenomenon had been observed previously with other nitro imidazole compound [18]. Thus we added drops of a strong alkaline solution to the nitro

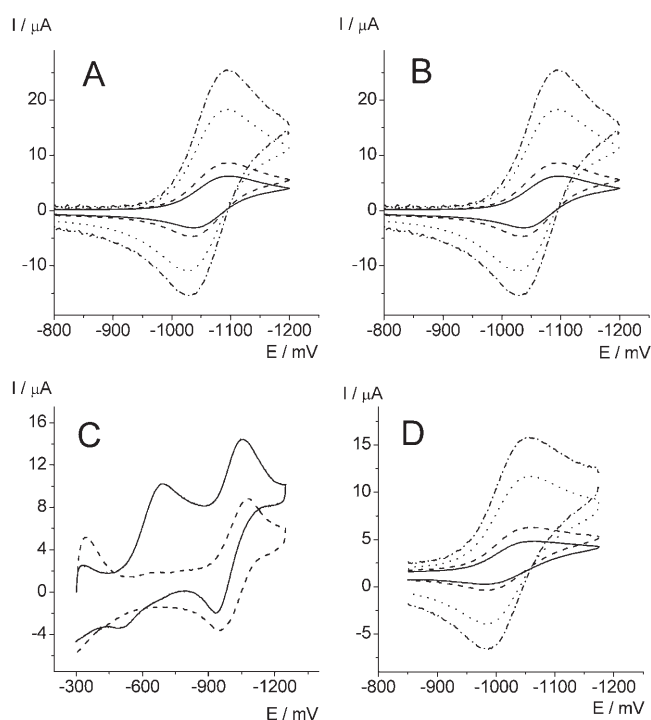


Fig. 8. Cyclic voltammograms at different sweep rates showing the reversible couple due to the one-electron reduction of M-NIm (A), M-I-NIm (B) at mercury electrode, in non-aqueous medium (100% DMF). C) Cyclic voltammograms of M-I<sub>2</sub>-NIm without added NaOH (solid lines) and with added NaOH drops (dashed line). D) Cyclic voltammograms at different sweep rates showing the reversible couple due to the one-electron reduction of M-I<sub>2</sub>-NIm with added NaOH.

compound solution in order to affect the equilibrium. In fact we saw the complete disappearance of the prepeak after alkalization of the medium (Fig. 8 D), nevertheless this effect was not reverted with the later addition of acid, as would expected if an acid-base equilibrium was present. Consequently, we discarded the existence of the acid-base equilibrium as the cause of producing the prepeak and probably the observed change in the prepeak is due to the occurrence of an irreversible chemical reaction whose elucidation needs further investigation. In the present study we have evaluated the  $\text{RNO}_2/\text{RNO}_2^{\cdot-}$  couple, without prepeak, i.e. after the addition of base generating a named OH-M-I<sub>2</sub>-NIm compound.

In Figure 8D the cyclic voltammogram of M-I<sub>2</sub>-NIm in presence of NaOH is displayed, showing a similar behavior with the sweep rate that M-NIm and M-I-NIm (Figs. 8A and B). From those experiments we calculated the stability of each nitro radical anion (Table 4). A completely different behavior between the compounds was found, being the radical generated from the two iodo substituted nitroimidazoles considerably more unstable than the M-NIm. Then it is possible to conclude that the iodo substitutions increase the nitro radical anion instability. The difference in the stability of the radicals was observed in this non aqueous medium, but not in mixed medium where the radicals decay with the same rate. This fact could be explained considering



Table 4. Peak potential values ( $E_p$ ) and decay constant ( $k_2$ ) for the nitro radical anion electrochemically formed in non-aqueous medium.

	M-NIm	M-I-NIm	OH-M-I <sub>2</sub> -NIm
$E_p$ (mV)	-1101	-1071	-1056
$E_7^1$ (mV) [a]	-458	-428	-413
$k_2 \times 10^{-2}$ [Ms] <sup>-1</sup>	5.81	132	1100

[a] vs. NHE

that the decay reaction in non aqueous medium is the dimerization of the radicals and not the disproportionation as in mixed medium.

Comparing the peak potential of the one electron nitro reduction ( $E_p$  values in Table 4) we can observe that the presence of iodo substituents in the molecule produces more easily reducible compounds, in a similar way that in protic and mixed media.

Under biological point of view the difference in the redox potential as function of the medium used to carried out the electrochemical experiments is relevant since that in several cases the redox potential of nitrocompounds, obtained in mixed or non-aqueous media, do not show correlation with biological activity. However, as has been previously described exists a good correlation between the cathodic peak potential, obtained in non-aqueous medium, with the  $E_7^1$  value obtained by pulse radiolysis [19]. The  $E_7^1$  value is a parameter that account 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 [17]. Consequently, with the experimental obtained values by cyclic voltammetry in non-aqueous medium we can calculate the  $E_7^1$  parameter for all the studied compounds. Table 4 lists the peak potential values obtained by cyclic voltammetry in non-aqueous medium and the calculated  $E_7^1$  values. This result is very significant because the obtained values for the  $E_7^1$  parameter are very closed with the described parameters of other nitroimidazole derivatives of biological significance such as metronidazole, ronidazole and tinidazole [17] meaning that our synthesized derivatives are also candidates to being enzymatically reducible.

#### 4. Conclusions

According to the above results we can conclude that all the studied compounds were reducible in protic, mixed and non-aqueous media at both, mercury and glassy carbon electrodes. However in the case of the iodo substituted derivatives a strong interaction with mercury in protic medium was found. This interaction was only partial in mixed medium and nonexistent in totally non-aqueous medium. According to the measurements on glassy carbon electrode we have found that the inclusion of iodo in the molecule produces a more easy reduction of the nitro group as expected from the electron withdrawing character of the iodo atom.

According to the voltammetrically obtained  $E_7^1$  values we can conclude that the nitro radical anion from all the studied compounds could be biologically formed via enzymatic reduction, because the energy requirements of reduction are similar with that obtained for other well-known enzymatic reducible nitroimidazole drugs.

Related to the formation and stability of the nitro radical anions it is possible to conclude that in protic medium (mercury electrode) only the M-NIm derivative produced radicals in the time schedule of the voltammetric experiment. From this results ( $k_2 = 2.2 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ ) and comparing the decay constant with that previously reported for 4-NIm ( $k_2 = 7.48 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ ) [16], using the same procedure, it is possible to conclude that the methyl substitution in position 1 of the imidazole ring produced a substantially more stable nitro radical anion. In mixed medium the radicals decay by a disproportionation reaction and its stability is not affected by the presence of iodo as substituent. However, in non-aqueous medium the nitro radical anion decays by a dimerization reaction and the stability is strongly diminished by the presence of iodo as substituent in the molecule.

#### 5. Acknowledgements

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