

Substituent Effect of 4-Nitroimidazole Derivatives: Acidic Hydrogen as Modulator of the Nitro Radical Kinetic Stability

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The electrochemical reduction of 1-methyl-4-nitro-2-carboxymidazole (RNO_2COOH) in a nonaqueous medium produced two reduction signals, which were influenced by the dissociation of the compound. The first reduction signal was caused by the reduction of the acidic species, and the second signal was a quasi-reversible couple caused by the reduction of the conjugate base to the radical dianion. According to the theory developed by Nicholson for the study of the electrode reaction kinetics, we have obtained the heterogeneous rate constant, $k^0 = (9.74 \pm 0.052) \times 10^{-3} \text{ cm s}^{-1}$, for the reduction of the conjugate base to the radical dianion. The voltammetric behavior of RNO_2COOH and its comparison with the behavior of another related compound such as 1-methyl-4-nitro-2-hydroxymethylimidazole is a good example to show the applicability of the general theory about the mechanism of self-protonation reactions in organic electrochemical processes to the specific case of nitroimidazole derivatives. According to the presented results, the presence of acidic hydrogen as a substituent in the nitroimidazole moiety would permit the modulation of the nitro radical anion stability, thus affecting its potential biological activity.

Nitroimidazole compounds have been widely used because of their properties as antibiotics, radiosensitizers, and antiprotozoans.¹⁻⁵ All the biological activity of these compounds depends on the reduction of the nitro group, which can accept up to six electrons to give the amine derivative.⁴ Consequently, only organisms that have redox mechanisms with a sufficient potential to reduce the nitro group produce the biologically active form of the compound. Although a detailed understanding of the mechanisms involving the biological activity of nitro compounds is still a matter of debate, there is no question that the use of these compounds in medicine and cancer therapy relies upon free-radical mechanisms. Specifically, the redox properties of the one-electron couple $\text{RNO}_2/\text{RNO}_2^-$ define virtually all the biological properties of these compounds.⁶ Consequently, the understanding of the nitro radical behavior is a permanent challenge for this type of compound, and the electrochemistry can play an important role in its study.

The electrochemical studies of 4-nitroimidazoles are rather scarce and restricted to a polarographic study of several 1,2-dialkyl-4-nitroimidazoles⁷ and some electrochemical studies on 4-nitroimidazole in both aprotic⁸ and protic media.⁹ More recently, we have synthesized and studied the electrochemical behavior of a 4-nitroimidazole substituted derivative, 1-methyl-4-nitro-2-hydroxymethylimidazole.¹⁰ From these studies, it is possible to conclude that the new 2-hydroxymethyl derivative of 4-nitroimidazole is more easily reducible than the parent 1-methyl-4-nitroimidazole and that its radical anion decays more rapidly than that of the parent nitroimidazole. Furthermore, it can be concluded that the electrode process is strongly dependent on both the nature of the medium (protic or aprotic) and on the substituents on the 4-nitroimidazole moiety.

In the scope of our current investigations to find pharmacologically important compounds that use the nitro radical anion as the active species, we have synthesized 1-methyl-4-nitro-2-carboxymidazole (RNO_2COOH) (Fig. 1), a 4-nitroimidazole derivative substituted in positions 1 and 2. In a previous study,¹⁰ we studied the nitro radical anion formation in aqueous, mixed, and nonaqueous media of the 1-methyl-4-nitro-2-hydroxymethylimidazole ($\text{RNO}_2\text{CH}_2\text{OH}$) (Fig. 1), but in this work we are interested in investigating the influence of the two-position substituent in the capability to produce nitro radical anions.

Consequently, the small number of reports on the electrochemical investigation of 4-nitroimidazole derivatives and the importance of its reduction in medical applications has stimulated us to carry out an exhaustive electrochemical study of 1-methyl-4-nitro-2-carboxymidazole in nonaqueous medium with the purpose of contributing to the knowledge of its redox behavior.

Experimental

Reagents and solutions.— RNO_2COOH (Fig. 1) was synthesized and characterized in our laboratory. All the other reagents employed were of analytical grade. Ultrapure water (18.2 M Ω cm) obtained from interchanged columns (Millipore Milli-Q system) was used.

Nitrogen gas was obtained from Alphagaz-Air Liquide with maximum impurities of $\text{H}_2\text{O} < 3 \text{ ppm}$; $\text{O}_2 < 2 \text{ ppm}$; $\text{C}_n\text{H}_m < 0.5 \text{ ppm}$.

All the voltammetric experiments were obtained after bubbling N_2 for 10 min in the cell before each run. The temperature was kept constant at $25 \pm 0.1^\circ\text{C}$ in all experiments.

Solutions for cyclic voltammetry were prepared starting from a 0.2 M stock solution of the nitroimidazole derivative in dimethyl sulfoxide (DMSO 99.9% pure obtained from Winkler, Ltd., Santiago, Chile) prepared daily by passing through a column of 0.4 nm molecular sieves. Final solutions in the voltammetric cell were prepared by diluting an appropriate quantity of the stock solution in order to obtain a final concentration of 1 mM.

Experiments were made in DMSO with 0.1 M tetrabutyl ammonium perchlorate (TBAP) as a supporting electrolyte.

Synthesis RNO_2COOH .— RNO_2COOH was synthesized and characterized in our laboratory starting from $\text{RNO}_2\text{CH}_2\text{OH}$, which was synthesized according to the previously published procedure.¹⁰ $\text{RNO}_2\text{CH}_2\text{OH}$ (1 g, 0.007 mol) was dissolved in hot water, and then drop-by-drop portions of sulfochromic mixture with continuous and vigorous stirring were added. The molar ratio for the oxidation was $\text{RNO}_2\text{CH}_2\text{OH}:\text{K}_2\text{Cr}_2\text{O}_7:\text{H}_2\text{SO}_4/1:4:3$. The oxidation mixture was refluxed for 12 h and then alkalized with KOH up to pH 7–8 and extracted with ethyl acetate. The organic extract was dried under vacuum. The product was recrystallized with activated charcoal in hot water, obtaining white crystals (55% yield). $^1\text{H NMR}$ (acetone d_6 , 300 MHz): 3.8 (s, 3H, $-\text{N}-\text{CH}_3$) 8.0 (s, 1H, $-\text{C} = \text{CH}-\text{N}-\text{CH}_3$). IR (KBr) ν_{max} : 3121, 1567, 1555, 1314.8, 1291.7. Elemental analysis for $\text{C}_5\text{H}_5\text{O}_4\text{N}_3$. Calculated: C: 35.10; H: 2.95; N: 24.56. Found: C:

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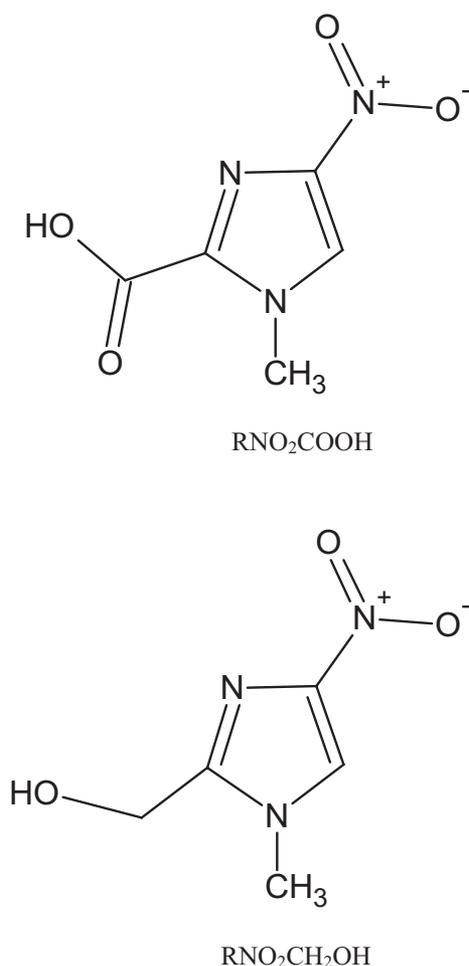


Figure 1. Molecular structures of RNO_2COOH and $\text{RNO}_2\text{CH}_2\text{OH}$.

36.90; H: 3.58; N: 25.52. Melting point: 134–137°C; R_f (ethyl acetate:butanol:acetic acid:water/80:10:5:5; silica gel 60 F, Merck) = 0.50.

Apparatus and methods.— Voltammetric curves were recorded on an electrochemical analyzer type BAS 100B/W (Bioanalytical System) attached to a PC computer with appropriate software (BAS 100W 2.3 for Windows) for total control of the experiments and data acquisition and treatment. A static mercury-drop electrode (BASi EF-1400) with a mercury-drop area of 0.43 mm^2 was used as the working electrode and a platinum wire (BASi MW-1032) as the counter electrode. All potentials were measured against $\text{Ag}|\text{AgCl}|\text{NaCl}$ (3M) (BASi MF-2052).

The standard heterogeneous charge transfer rate constant (k^0) associated with the redox process of Eq. 9 was calculated from the peak-to-peak separation (ΔE_p) of the cyclic voltammogram according to the method described by Nicholson.¹¹ (All the ΔE_p measurements were IR compensated with positive-feedback circuitry to make sure of a minimized uncompensated resistance.) E_λ was selected at least $90/n$ mV beyond the cathodic peak.

UV-visible spectra were recorded in the 200–600 nm range using a Unicam UV-3 spectrophotometer. Temperature was controlled with a thermostat/cryostat Selecta Frigiterm-10 with a precision of $\pm 0.1^\circ\text{C}$. The synthesized compound was characterized by ^1H NMR spectrometry using a 300 MHz spectrometer (Bruker, WM 300) and an elemental analyzer (Perkin-Elmer, 240 B).

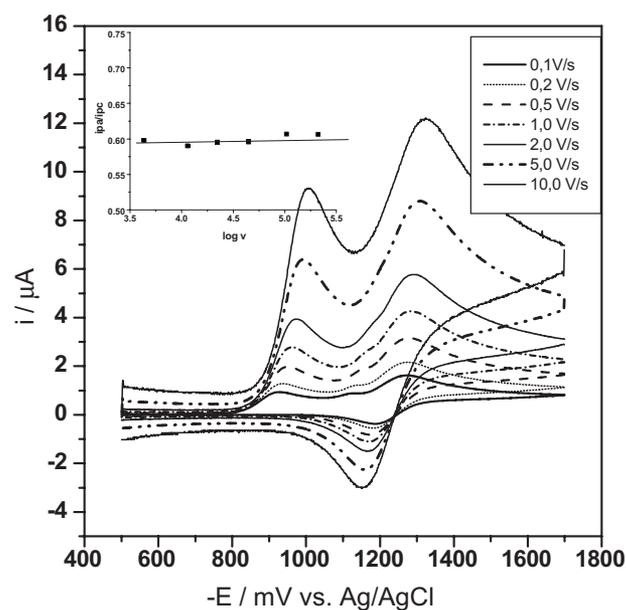


Figure 2. Cyclic voltammograms of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP at different sweep rates. (Inset) The $i_{p,a}/i_{p,c}$ at each scan rate.

Results and Discussion

Cyclic voltammograms of RNO_2COOH at the hanging mercury-drop electrode were recorded in anhydrous DMSO + 0.1 M TBAP. As observed in Fig. 2, two main cathodic peaks are displayed. The first cathodic peak has a potential peak value, $E_{p,c}$, of -0.95 V and the second one an $E_{p,c}$ value of -1.33 V , at a sweep rate of 1 V/s. The more cathodic of these peaks shows a corresponding anodic peak with an anodic peak potential, $E_{p,a}$, of -1.15 V at a sweep rate of 1 V/s. Thus, the second cathodic peak forms a voltammetric couple with the anodic peak, showing a difference between cathodic and anodic peak potentials, ΔE_p , of approximately 180 mV. Also, it can be observed from the inset of Fig. 2 that the current ratio, $i_{p,a}/i_{p,c}$, is different from 1 at different sweep rates, showing that a totally reversible process must be discarded.

Cyclic voltammograms of the isolated first peak at different sweep rates are shown in Fig. 3. From these voltammograms, we obtained a linear relation between the cathodic peak current and the square root of the sweep rate as shown in the plot of Fig. 4. This linear dependence is indicative of a diffusion-controlled process. In the other plot of Fig. 4, a linear dependence between the peak potential of the first cathodic peak and the logarithm of the sweep rate with a slope of 0.035 V is shown. From both plots in Fig. 4, we conclude that the first peak would obey to a diffusion-controlled process wherein a slow one-electron process is followed by a fast chemical reaction according to the following reaction scheme



wherein RNO_2COOH represents the acidic species of 1-methyl-4-nitro-2-carboximidazole and $(\text{RNO}_2\text{COOH})^-$ represents its corresponding nitro radical anion. The above reaction scheme corresponds to a coupled process wherein the nitro radical anion generated in the first step (Eq. 1) decays chemically in a second step (Eq. 2). Considering that the chemical step is fast compared with the one-electron transfer, on back oxidation of the nitro radical anion is possible, thus producing the voltammogram of irreversible aspect displayed in Fig. 3.

The chemical step would most likely include the decay of the nitro radical anion by some reaction such as protonation, dimerization, or a father-son-type reaction, as has previously been described

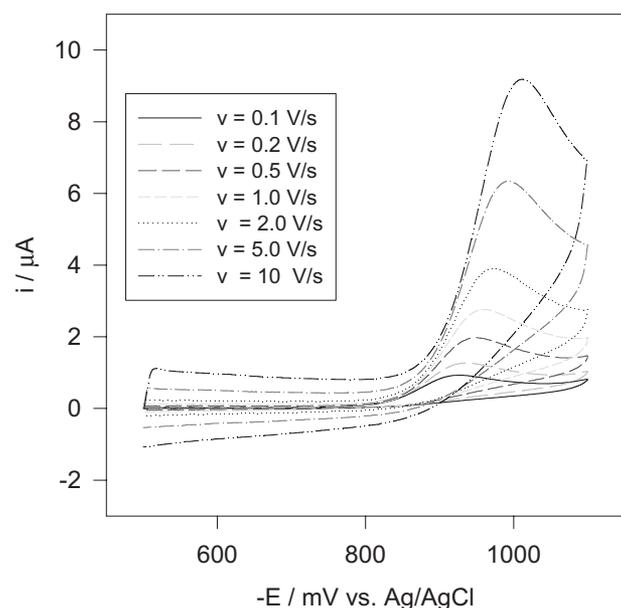


Figure 3. First peak cyclic voltammograms of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP at different sweep rates.

for organic electrochemical process¹² or, in particular, for nitro compounds.^{8,9} Vianello⁸ described a father–son-type reaction for 4-nitroimidazole in aprotic medium, but he discarded the autoprotonation for N(1) methyl 4-nitroimidazole substituted derivatives.

In order to resolve this question we have carried out the following experiments. Figure 5 shows the effect of adding different quantities of a base as tetrabutylammonium hydroxide (TBA-OH) on a solution containing 1 mM of RNO_2COOH . According to this result, it is possible to conclude that the addition of the base produces a decrease in the first cathodic peak, which means a decrease in the concentration of the initial electroactive reactant (RNO_2COOH). Furthermore, this behavior is reverted with the addition of acid, as is shown in the experiment displayed in Fig. 6. In fact, the addition of an acid as HClO_4 (in ethanol) over the previous alkalinized solution produced the reversion of the reaction toward the regeneration of the electroactive reactant (RNO_2COOH). The above experiments displayed in Fig. 5 and 6 can be explained assuming the existence of dissociation equilibrium between the acidic species and the corresponding conjugate base according to



wherein the carboxylate species RNO_2COO^- represents the conjugate base formed as a consequence of the loss of the acidic proton on the carboxylic substituent in 2-position of the nitroimidazole ring in the alkalinized solution.

Considering the equilibrium of Eq. 3, we can explain the two cathodic peaks for the reduction of this 4-nitroimidazole derivative. The electroactive species producing the first cathodic irreversible peak corresponds to the acidic species RNO_2COOH , and the electroactive species producing the second cathodic peak corresponds to the conjugate base RNO_2COO^- . This peak assignment is in accord with the general rule that a conjugate base is reduced at more negative potentials than the corresponding acid form; in other words, the conjugate base is more difficult to reduce than the acid form. Furthermore, a decrease in the first cathodic peak was observed when successive sweeps were carried out. In Fig. 7, we can observe three successive scans on the same surface at 0.2 and 0.5 V/s. This fact implies that the electrolysis procedure generated a species (nitro radical anion) that acts as a base, deprotonating the parent compound in a father–son-type reaction according to the following scheme

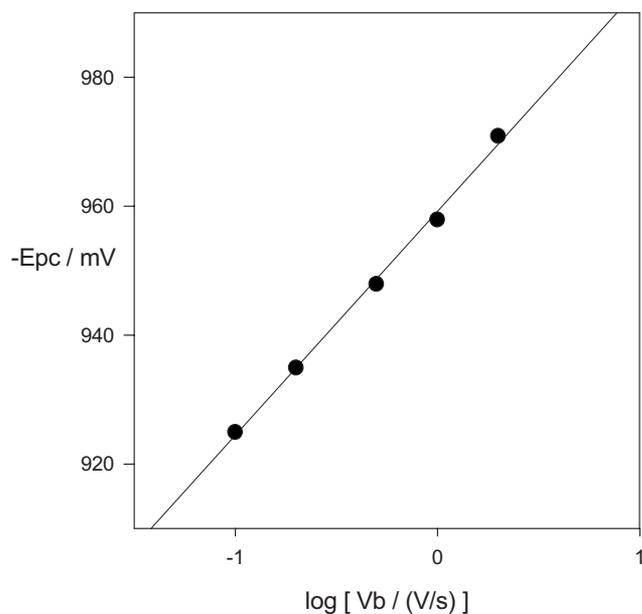
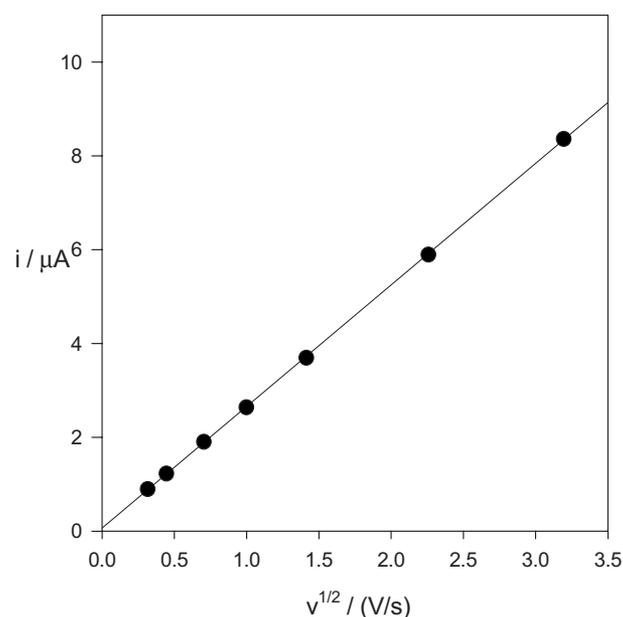
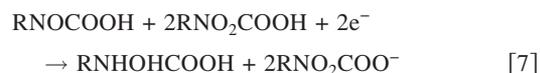


Figure 4. $i_{p,c}$ vs $v^{1/2}$ and $E_{p,c}$ vs $\log v$ plots of the first peak of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP.



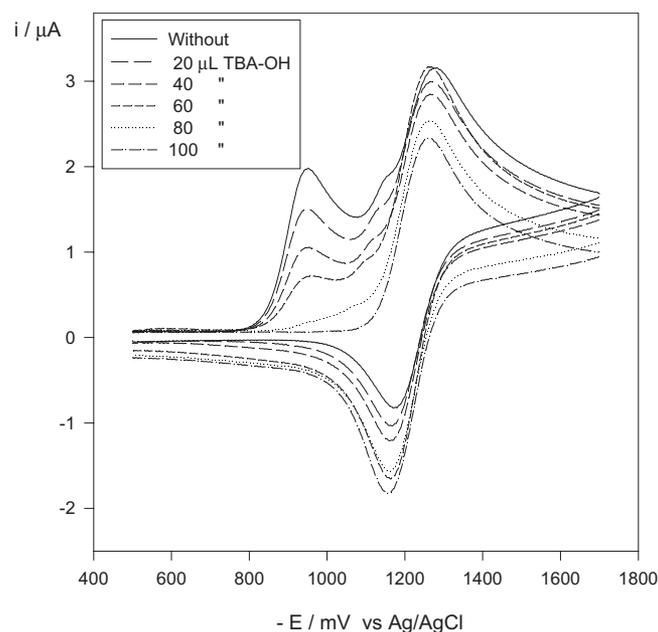


Figure 5. Cyclic voltammograms of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP with different quantities of 0.1 M TBA-OH added up to 100 μL . $v = 0.5 \text{ V/s}$.



This mechanism was adapted from the previously described general behavior in organic electrochemical processes in the seminal paper from Amatore et al.¹¹ and specifically from the 4-nitroimidazole behavior from Roffia et al.⁸

The intermediate shoulders, clearly present in voltammograms of Fig. 5 and 6, can be explained by the reduction of the intermediate species such as the conjugate base of the nitroso derivative. Consid-

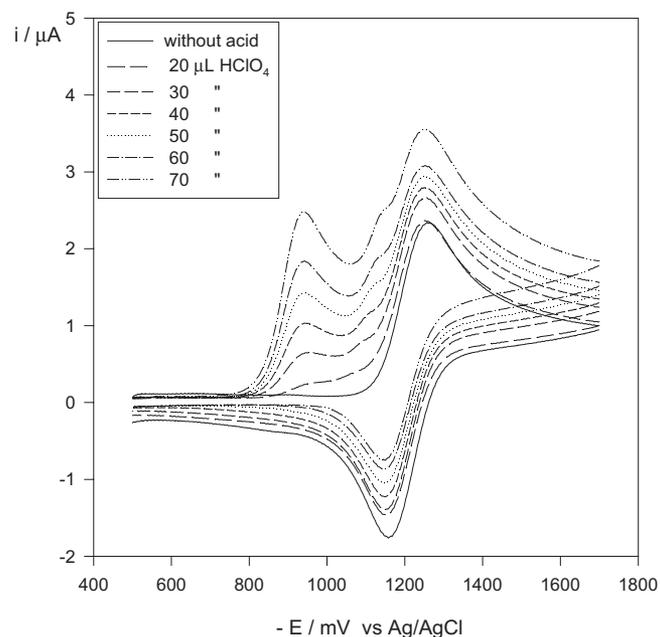


Figure 6. Cyclic voltammograms of an alkalized solution (100 μL of 0.1 M TBA-OH) of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP with different quantities of 0.2 M HClO_4 (in ethanol) added. $v = 0.5 \text{ V/s}$.

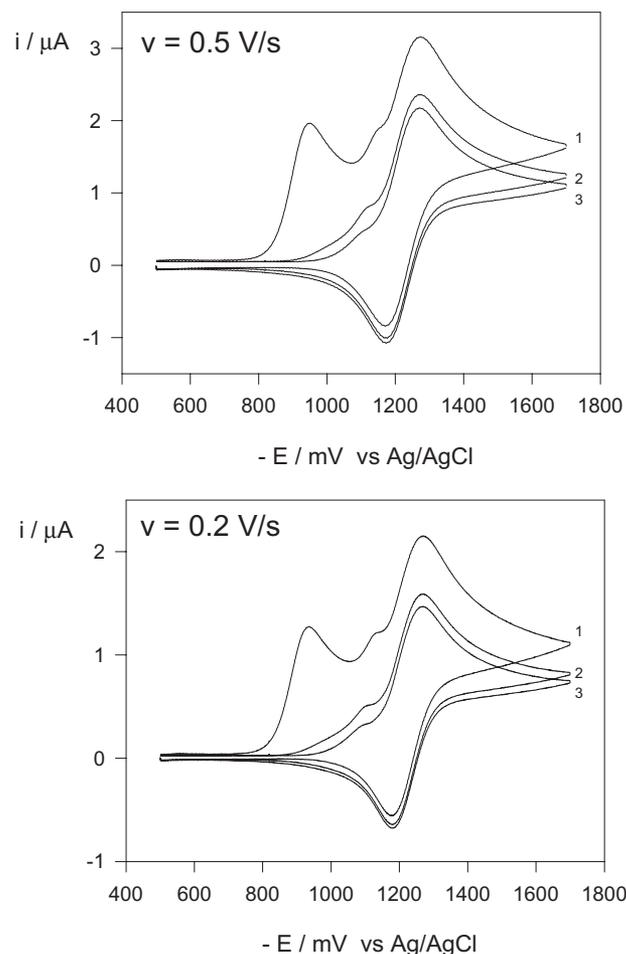


Figure 7. Successive cyclic voltammograms of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP. 1 = 1st scan, 2 = 2nd scan, and 3 = 3rd scan. $v = 0.5$ and 0.2 V/s .

ering the above results and previous information, the first irreversible and diffusion-controlled peak can be ascribed to the irreversible reduction of the RNO_2COOH species obeying the above overall Reaction 8.

The second peak can be studied in isolation only in the case of solutions with TBA-OH. When a sufficient quantity of base is added, the only species in the solution is the conjugate base (according to the equilibrium of Eq. 3). Consequently, the voltammogram (Fig. 8) shows only a cathodic peak at -1.35 V (2.0 v/s), with a corresponding anodic peak in the back sweep. The cathodic peak is due to the reduction of the conjugate base to produce the corresponding nitro radical anion of the conjugate base (or radical dianion species) according to



The return-to-forward peak current ratio $i_{p,a}/i_{p,c}$ and the ΔE_p values for the couple conjugate base/dianion were measured for each single cyclic voltammogram at different sweep rates. The separation between the anodic and cathodic peaks was larger than that expected for a reversible one-electron process of a freely diffusing species, indicating that the redox reaction of the conjugate base/dianion couple in this medium is only quasi-reversible. We have carried out an exhaustive determination of ΔE_p values at different scan rates, finding a linear behavior between ΔE_p and the square root of the scan rate (Fig. 9) which agrees well with the theory for a typical quasi-reversible process.¹³ The quasi-reversibility for this process was also confirmed from the $i_{p,c}$ vs $v^{1/2}$ plot (Fig. 9), which exhibits

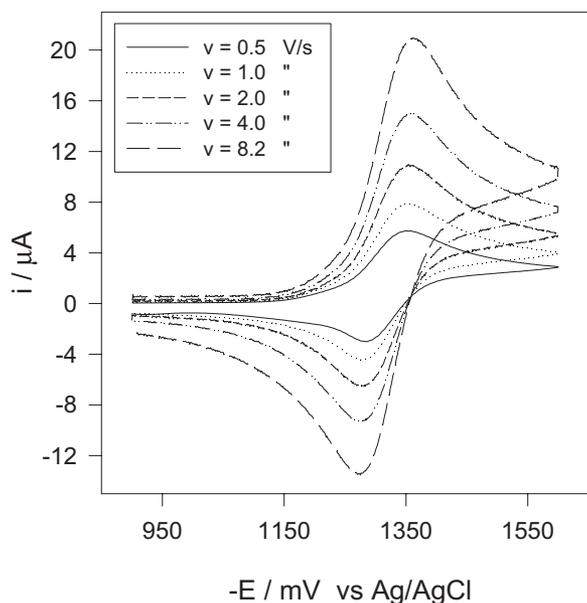


Figure 8. Cyclic voltammograms of 1 mM RNO_2COOH in DMSO and 0.1 M TBAP alkalized with 120 μL of 0.1 M TBA-OH at different sweep rates.

a linear trend when it is plotted in a narrow range of scan rates.¹¹ With the above results, we can be sure that any possible uncompensated resistance is sufficiently small that the resulting voltage drops are negligible compared to the ΔE_p attributable to kinetic effects. Under these conditions of quasi-reversibility, it may be possible to calculate the heterogeneous charge-transfer rate constant, k^0 , from the ΔE_p values according to the procedure described by Nicholson.¹¹ These ΔE_p values were introduced in a working curve for obtaining the transfer parameter, ψ , and then the k^0 value for the electron-transfer process using the previous equation described by Bontempelli¹⁴ and Ramamurthy.¹⁵ According to this equation we have obtained a linear relation between ψ and $v^{-1/2}$ as shown in Fig. 9. From the slope of such a line, a k^0 value of $(9.74 + 0.052) \times 10^{-3} \text{ cm s}^{-1}$ for the one-electron reduction of the conjugate base to form the radical dianion was obtained.

Summarizing the above results, we can conclude that in the case of the RNO_2COOH derivative, the nitro radical anion was not able to be kinetically stabilized in nonaqueous medium. The term kinetic stability is referred to the reactivity of the radical anion with a proton donor and not to the thermodynamic stability. The carboxylic group at position 2 being conjugated with the nitro group at position 4 does stabilize (thermodynamically) the radical anion by delocalization of the spin (and the charge) onto the carboxyl moiety (a carboxylate group would delocalize the spin, too).

The above obtained results for the electrochemical behavior of RNO_2COOH differ significantly with the previously obtained re-

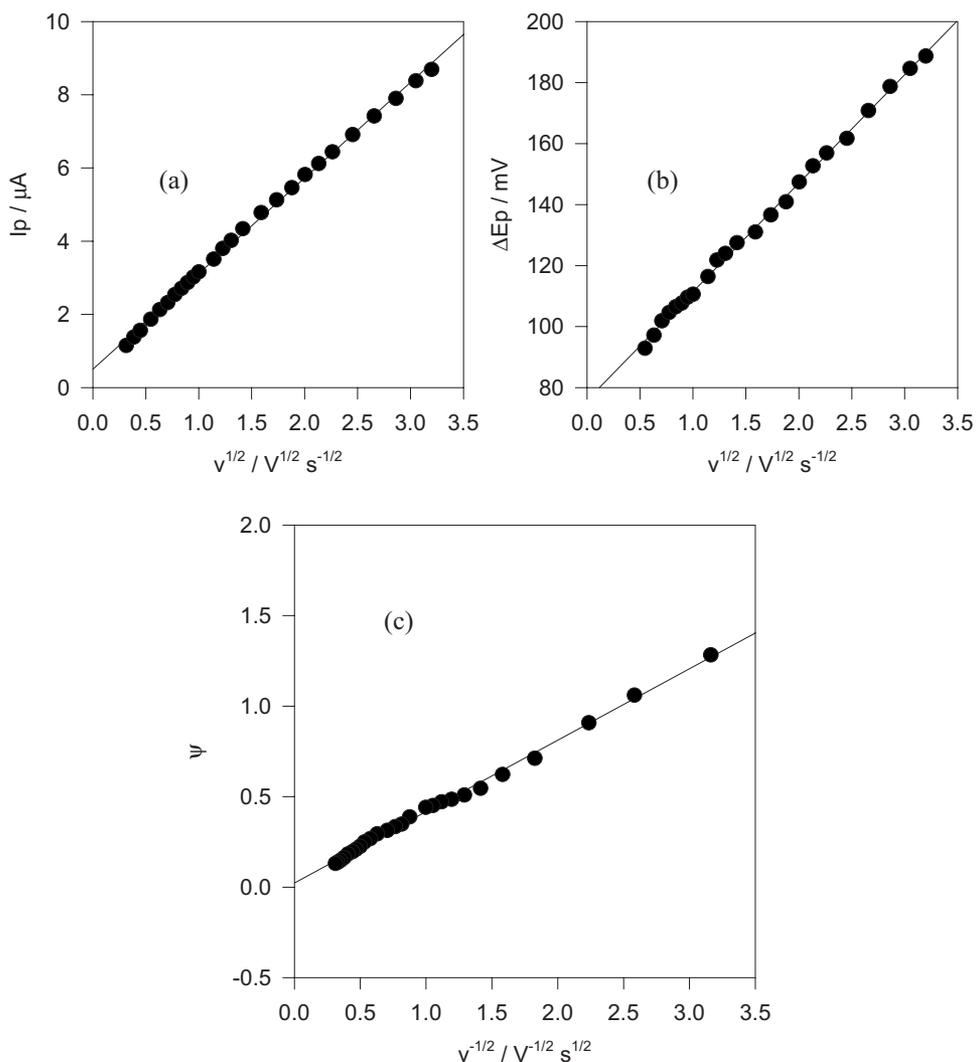


Figure 9. Cathodic peak current and ΔE_p vs square-root scan-rate plots, and the transfer parameter, ψ , vs the inverse of square-root scan-rate plot obtained from solutions containing 1 mM RNO_2COOH in DMSO and 0.1 M TBAP alkalized with 120 μL of 0.1 M TBA-OH.

sults for the related compound $\text{RNO}_2\text{CH}_2\text{OH}$,¹⁰ wherein the nitro radical anion was easily generated and kinetically stabilized in mixed and nonaqueous media.

Our results show that the change of COOH by CH_2OH in the 2-position of the 1-methyl-4-nitroimidazole ring produces a significant change in the nitro reduction mechanism. The main difference between both substituents in 2-position is the acidity level of the hydrogen, with the hydrogen of the carboxylic group being a lot more acidic than the alcoholic hydrogen. Consequently, in this case, the acidic character of the hydrogen in 2-position turned out to be decisive in the change of the mechanism.

The most related previous papers^{8,9} describing the reduction of 4-nitroimidazole and 2-nitroimidazole in nonaqueous medium show a similar behavior but in such cases the acidic hydrogen corresponded to the hydrogen atom attached to the imino nitrogen on the nitroimidazole ring. In this paper we have shown that the same phenomenon can be obtained with acidic hydrogen not necessarily attached directly on the nitroimidazole ring, thus permitting the modulation of the nitro radical-anion stability with several different substituents.

The obtained results are consistent with the acidity of the substituent at position 2. The $\text{p}K_a$ of imidazole itself in DMSO is 19,¹⁶ so the $\text{p}K_a$ of nitroimidazole in DMSO is probably in the 13–15 range based on a comparison with the $\text{p}K_a$ of p-nitrophenol, 11, and that of phenol, 16; the $\text{p}K_a$ of p-nitrobenzoic acid in DMSO is 9, and that of RNO_2COOH should be similar (conjugation between the carboxylic group and the nitro group). So, it is totally expected that a father–son reaction would be observed in the reduction of RNO_2COOH in a dipolar aprotic solvent. However, the $\text{p}K_a$ of an alcohol in DMSO is in the 29–30 range,¹⁶ and it is therefore expected that no father–son reaction would occur in the reduction of $\text{RNO}_2\text{CH}_2\text{OH}$.

Considering that the nitro radical anion is responsible for the biological activity of these compounds, the possibility to modulate its kinetic stability could have an impact on the corresponding biological activity.

Conclusions

We have found that the reduction of RNO_2COOH in nonaqueous medium is strongly determined by the existence of an acid-base equilibrium, with both acidic and conjugate base species coexisting. The nitro radical anion from RNO_2COOH was not kinetically sta-

bilized in nonaqueous medium due to the existence of a father–son-type reaction between the nitro radical anion and the parent compound.

By comparison of the electrochemical behavior of RNO_2COOH with $\text{RNO}_2\text{CH}_2\text{OH}$, we concluded that the existence of acidic hydrogen in the substituent produces a notorious change in the mechanism, generating kinetic destabilization of the nitro radical anion of the 4-nitroimidazole derivative.

The inclusion of substituents in the nitroimidazole moiety containing hydrogen with different acidity levels would permit the modulation the nitro radical anion kinetic stability, thus modulating its biological activity.

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