Voltammetric Behavior of a 4-Nitroimidazole Derivative

Nitro Radical Anion Formation and Stability

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A new synthesized compound, 1-methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH), was electrochemically reduced at the mercury electrode in aqueous, mixed, and aprotic media. In an aqueous medium, only one voltammetric peak was observed because of the four-electron, four-proton reduction of the nitro group to the hydroxylamine derivative in the 2-12 pH range. For the mixed and nonaqueous media, it was possible to observe a reversible couple due to the first one-electron reduction step of the nitro group to the nitro radical anion.The nitro radical anion decays by a disproportionation reaction in mixed media and by dimerization in a nonaqueous medium. Both disproportionation and dimerization rate constants, k_2, were determined according to Olmstead's approach, obtaining a value of 1460 ± 110 M^{-1} s^{-1} in aprotic medium. In mixed media, the values were dependent both on pH and on the nature of the cosolvent.After comparison of 4-MNImOH with the parent compound, 4-nitroimidazole, we concluded that the substitution with 1-methyl and 2-hydroxymethyl produces a more easily reducible nitro compound and a less stable nitro radical anion than the unsubstituted 4-nitroimidazole. According to the electrochemical results, the 4-MNImOH derivative would be more suitable for enzymatic reduction and less toxic to the host than 4-nitroimidazole.

In the last decades, nitroimidazoles have been the source of many investigations because of their properties as antibiotics, radiosensitizers, and antiprotozoans.[1-3] The biological activity of nitroimidazoles is dependent on the nitro group reduction process due to the formation of active intermediate species that interact with DNA and cause biochemical damage. The reduction of these compounds can follow two different routes depending on whether the medium is aerobic or anaerobic,[4,5] however, both routes share a common first step, *i.e.*, the one-electron reduction of the nitro group to form the nitro radical anion ($RNO_2^{\bullet -}$). Consequently, $RNO_2^{\bullet -}$ is a key intermediate in the biological activity, and the understanding of its behavior is a permanent challenge for these type of compounds.

Three types of nitroimidazole derivatives have been currently used, namely, 2-, 4-, and 5-nitrosubstituted derivatives; however, there are still no conclusive results about the incidence of the nitro substitution in their biological activity. A study on the reduction of 2-, 4- and 5-nitroimidazole drugs by hydrogenase 1 in *Clostridium pasteurianum*[6] revealed that the rate of reduction of the nitroimidazole compounds correlated with their one-electron reduction potential. However, the reduction rates for the drugs did not correlate with the antibacterial activity against *Clostridium pasteurianum*, suggesting that other factors are also important for determining the antimicrobial potencies of these compounds. Another study on the activity of nitroimidazoles against *Trichomonas vaginalis*[7] revealed that the potency of this activity follows the order 5-nitroimidazole $>$ 2-nitroimidazole $>$ 4-nitroimidazole. However, the description of the mutagenic and carcinogenic properties of some 2- and 5-nitroimidazoles have increased interest in the minor mutagenic 4-nitroimidazoles.[8-14]

The electrochemical studies of nitroimidazoles are mainly focused on the analytical determination of some pharmacologically important 5-nitroimidazoles, such as metronidazole, ornidazole, secnidazole, tinidazole, and megazol.[15-19] In addition, cyclic voltammetric studies of nitro radical anions produced from 5-nitroimidazole derivatives have been reported, demonstrating the usefulness of this technique to the study of nitro free radicals.[20-24] However, electrochemical studies of 4-nitroimidazole derivatives are scarce and restricted to a polarographic study of several 1,2-dialkyl-4-nitroimidazoles[25] and some electrochemical studies on the cyclic voltammetric behavior of 4-nitroimidazole in aportic[26] and protic media.[27] The results obtained from these studies differ widely, depending on the protic or aprotic medium. The one-electron reduction of 4-nitroimidazole in protic medium at alkaline pH produces a stable nitro radical anion on the time scale of the cyclic voltammetry. But, in aprotic medium, the nitro radical anion cannot be stabilized because of a rapid decay of the nitro radical produced by a fast protonation reaction by the starting 4-nitroimidazole (father-son type reaction).

In the scope of our current investigations to find new pharmacological important compounds that use the nitro radical anion as the active specie, we have synthesized 1-methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH) (Fig. 1), a new 4-nitroimidazole derivative substituted in positions 1 and 2. Our first aim is studying its electrochemical behavior with the focus on the formation and stability of the nitro radical anion in aqueous, mixed, and nonaqueous media. This is our first attempt to reveal the incidence of different substitutions in the 4-nitroimidazole ring to find nitro radical anions with improved pharmacological potency.

Experimental

Reagents and solutions.— 4-MNImOH was synthesized and characterized in our laboratory. All the other reagents employed were of analytical grade. Ultrapure water (18.2 MΩ cm) obtained from interchanged columns (Millipore Milli-Q system) was used.

Stock solutions of 4-MNImOH were prepared at a constant concentration of 10^{-2} M in ethanol. The polarographic and cyclic voltammetric working solutions were prepared by diluting the stock solution until final concentrations of 0.1 or 1 mM were obtained. The dilution solutions were Britton-Robinson buffer (0.1 M) for aqueous media, a mixture of 30/70: ethanol/Britton-Robinson buffer (0.1 M) or 60/40: dimethylformamide (DMF)/citrate buffer (0.015 M) KCl (0.3 M) for mixed media, and 100% DMF containing 0.1 M tetrabutylammonium perchlorate (TBAP), as supporting electrolyte, for nonaqueous solvent. The pH was adjusted with small aliquots of concentrated NaOH or HCl, respectively, in the aqueous and mixed solvents. All the polarographic experiments were obtained after a purge with N_2 for ten min in the cell before each run. All the experiments were carried out at room temperature.

Synthesis of 4-MNImOH.— 4-MNImOH (10 g, 0.09 mol) and potassium nitrate (20.5 g, 0.20 mol) were mixed and added slowly to sulfuric acid (5.4 mL, 0.10 mol) taking care not to exceed 50°C. The mixture was then maintained in a hot water bath for 3 h and stirred for 15 h at room temperature. The solution was carefully neutralized with sodium hydrogen carbonate. The product was extracted with ethyl acetate. The white solid was desiccated in vacuum. The yield of the recrystallized product in ethanol was 40%. ^{1}H NMR (300 MHz, D_2O): δ 3.2 (s, 1H, — OH) 3.8 (s, 3H, —N — CH_3) 4.5 (s, 2H, —CH_2—) 8.0 (s, 1H, —C ═ CH — N — CH_3) ^{13}C NMR (75 MHz, D_2O): 30.0 (—CH_3). 55.0 (—CH_2 — OH). 120.0 (O_2N — C ═ CH — N — CH_3). (2 × 149.8) (O_2N

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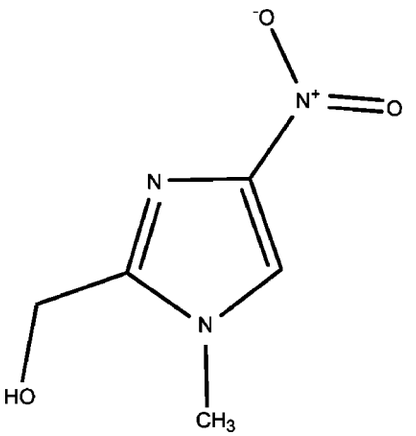

Figure 1. Chemical structure of 1-methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH).

— C(═CH) — N ═ C — CH_2OH). Elemental analysis for $C_5H_7O_3N_3$. Calculated: C: 38.22; H: 4.49; N: 26.74. Found: C: 37.90; H: 4.58; N: 26.52. melting point: 180-181°C Rf(benzene:methanol:acetic acid/45:8:1; Silica Gel 60 F, Merck) = 0.38

Apparatus.— Electrochemical experiments, differential pulse polarography (DPP), tast polarography, coulometry, and cyclic voltammetry were performed with a totally automated BAS-100 voltammetric analyzer attached to a PC with proper BAS 100W version 2.3 software for total control of the experiments and data acquisition and treatment. A controlling growth mercury electrode (CGME) polarographic stand was used with a dropping mercury electrode (DME) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode. For differential pulse (DP) and tast polarography, the CGME stand was used in a CGME mode and for cyclic voltammetric experiments a static mercury drop electrode mode was used.

Spectrophotometric measurements were carried out with an ATI Unicam Model UV3, UV-vis spectrophotometer using a 1 cm quartz cell and equipped with a PC with the Vision acquisition and treatment program.

All pH measurements were carried out with a WTW microprocessor-controlled standard pH ion meter pMX 3000/pH equipped with a glass pH-electrode Sen Tix 81. The standard solutions used for calibration were WTW 4.006, 6.865, and 9.180. Measurements of pH were corrected according to the following equation:[28] $pH^* - B = \log U^o_H$, where pH^* equals $-\log a_H$ in the mixed solvent, B is the pH meter reading, and the term $\log U^o_H$ is the correction factor for the glass electrode, which was calculated from the different mixtures of DMF and aqueous solvent, according to a procedure reported previously.[29]

Methods.—*Polarography*.—Differential pulse polarograms were carried out with a DME under the following operating conditions: scan rate 4 mV/s, pulse amplitude 50 mV, pulse width 50 mV, sample width 17 ms, and drop time 1000 ms. TAST polarograms (TPs) were carried out using a DME with the following operating conditions: scan rate 4 mV/s, sample width 17 ms, and drop time 1000 ms.

Cyclic voltammetry.—For the kinetic analysis carried out in alkaline pH, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible one-electron couple ($ArNO_2/ArNO_2^{\bullet -}$) was measured for each cyclic voltammogram (CV) according to the procedure described by Nicholson.[30] The scan rate (υ) ranged from 0.1 to 10 V s^{-1}. Using the theoretical approaches of Olmstead *et al.*,[31,32] the I_{pa}/I_{pc} values measured experimentally at each scan rate were inserted into a working curve to determine the parameter ω, which incorporates the effects of the rate constant, nitro compound concentration, and scan rate. A plot of ω *vs.* τ 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 chemical reaction of $ArNO_2^{\bullet -}$, C^o is the nitro compound concentration, $\tau = (E_\lambda - E_{1/2})/\upsilon$, E_λ is the switching potential, and $E_{1/2}$ is the cyclic voltammetric half-wave potential; and υ is the sweep 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 ω *vs.* τ. The assumption that the decomposition of $ArNO_2^{\bullet -}$ follows second-order kinetics is supported by the linear relation between the kinetic parameter ω and the time constant τ.

Considering that the decay of $ArNO_2^{\bullet -}$ follows second-order kinetics, it is possible to calculate the half-lifetime, $t_{1/2}$, from the well-known equation, $t_{1/2} = (k_2 C^o)^{-1}$.

Coulometry.—Coulometry was carried out on a mercury pool electrode in 0.1 M Britton-Robinson buffer/ethanol 70/30 at pH 4 and 7. The potential applied was −530 and −676 mV for pH 4 and 7, respectively. Oxygen was removed with pure and dry presaturated nitrogen. A three-electrode circuit with an Ag/AgCl electrode was used as reference and a platinum mesh as a counter electrode. A BAS-CV 50 assembly was used to electrolyze the compound.

Solutions containing an accurately weighed amount of 4-MNIm-OH were subjected to successive short electrolysis for 6 min to ensure that all the original compound had been consumed. This procedure was followed until the charge obtained was equal to the background charge. The net charge was calculated by correcting the estimated background current. The total net charge corresponds to the sum of all the individual processes. The number of electrons is obtained by application of the well-known Coulomb law

$$Q = nFe \qquad [1]$$

where, Q = total net charge, n = mole number of electroactive specie in solution, F = Faraday's constant, and e = the number of electrons. The number of electrons was obtained after five runs at each pH.

Spectrophotometry: determination of pK'_a.—The sensitivity of the band at 304 nm with pH was used to determine the spectrophotometric apparent pK'_a. The pH solution was changed each 0.5 unit. The temperature was kept constant at 25°C. The concentration was 1×10^{-4} M for the entire pH scale. The value of pK_a' obtained was calculated by the linear regression method.[33]

Simulations.—Simulated CV curves were obtained by using the DIGISIM 2.1 CV simulator for Windows software (BAS, USA). The software was run using a Gateway 2000 PC.

Electrolysis: Electron spin resonance (ESR) measurements.—The ESR spectra from the nitroimidazole derivative were recorded *in situ* in the cavity of a Bruker ECS 106 spectrometer with 100 kHz field modulation at microwave band X (9.68 GHz) and at room temperature. The hyperfine splitting constants were considered to be accurate within 0.05 G. The electrolysis was performed by reduction at −900 mV in the ESR cell using a platinum wire electrode and an $Ag/AgCl/KCl_{sat}$ reference electrode. The concentration of the 4-MNImOH was 5 mM in DMF with 0.1 M of TBAP as supporting electrolyte.

Results and Discussion

The new synthesized compound, 4-MNImOH, was electrochemically reduced at the mercury electrode in different media, but its reduction was strongly affected by solvent and pH changes. In a totally aqueous medium containing 100% Britton-Robinson 0.1 M, only one signal in all the pH scale was detected (Fig. 2a). In the polarograms (mainly at acid pH) one can observe a polarographic maximum, probably because of the adsorption of the 4-MNImOH or some reaction product on the mercury surface. This assumption was

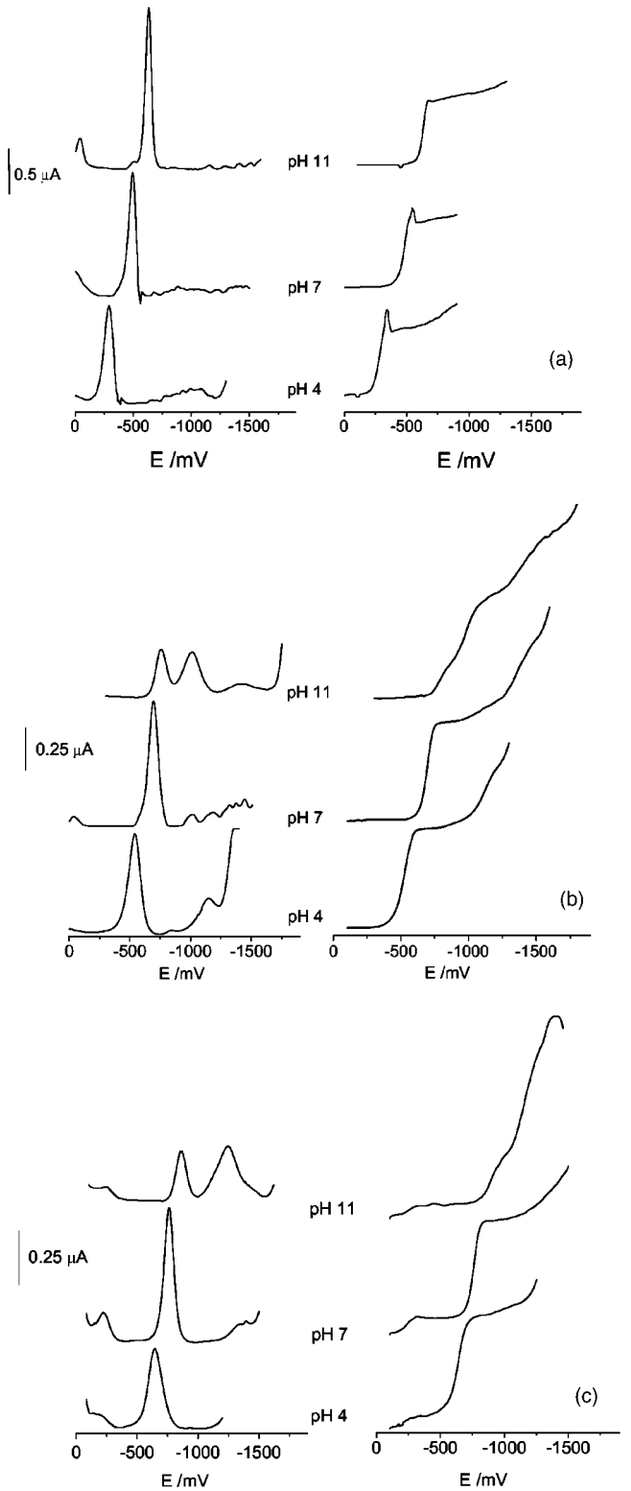

Figure 2. Differential pulse and tast polarograms of 4-MNImOH in (a) aqueous (0.1 M, Britton-Robinson buffer) and mixed: (b) DMF/citrate and (c) ethanol/Britton-Robinson buffer media at different pH.

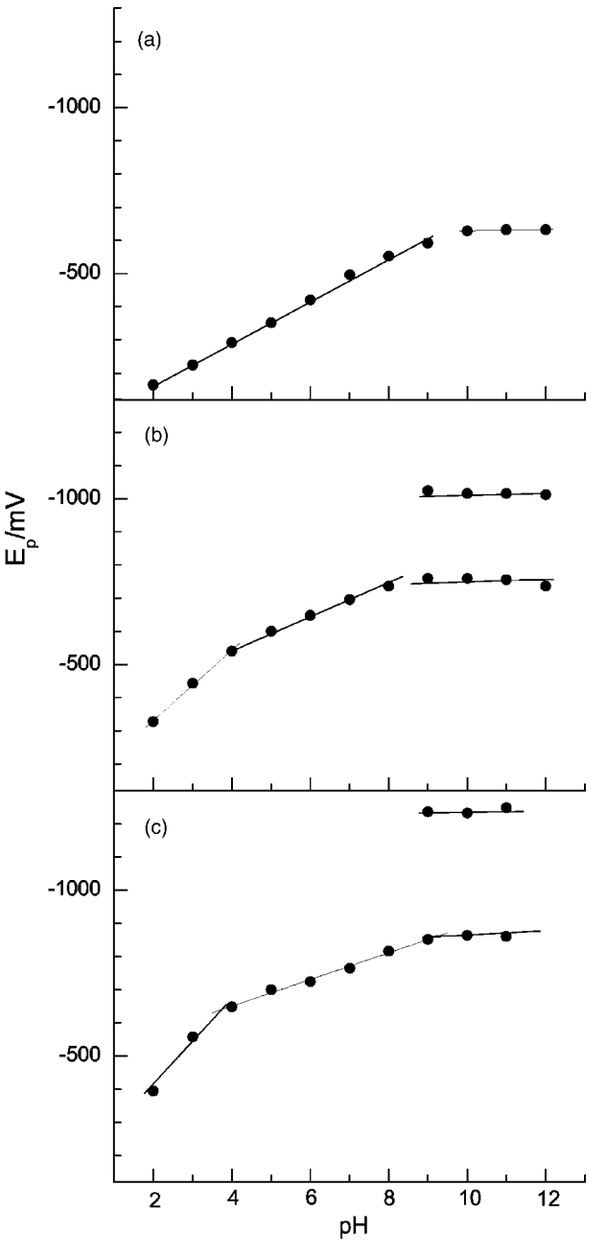

Figure 3. Peak potential dependence with pH in (a) aqueous medium (0.1 M, Britton-Robinson buffer) and mixed media: (b) DMF/citrate and (c) ethanol/Britton-Robinson buffers.

corroborated by electrocapillary curves (data not shown). Consequently, to avoid adsorption problems, we changed the electrolyte from aqueous to mixed media, adding a cosolvent to the buffer, *i.e.*, ethanol or DMF. As observed in Fig. 2b, the addition of a cosolvent permitted us to eliminate the polarographic maxima by avoiding adsorption problems in the electrodic process.

For mixed media, using either ethanol or DMF as cosolvent, the behavior was different, showing two peaks (or waves) at alkaline pH (Fig. 2b and c). In this medium, the peak appearing at acid pH splits into two peaks at pH $>$ 8. Under all conditions, the signal was pH dependent only up to pH 8, as observed in the potential peak *vs.* pH plot displayed in Fig. 3. At pH values beyond 8, the signals were pH independent, showing a change in the mechanism, meaning that there are no protons involved before the rate-determining step. Furthermore, the limiting currents obtained by tast polarography were pH independent, claiming a diffusion-controlled process.

From the E_P *vs.* pH plot, we can observe two different breaks: First, the previously mentioned break at pH 8 as a consequence of the change of mechanism and second the break at pH 3.5 due to the

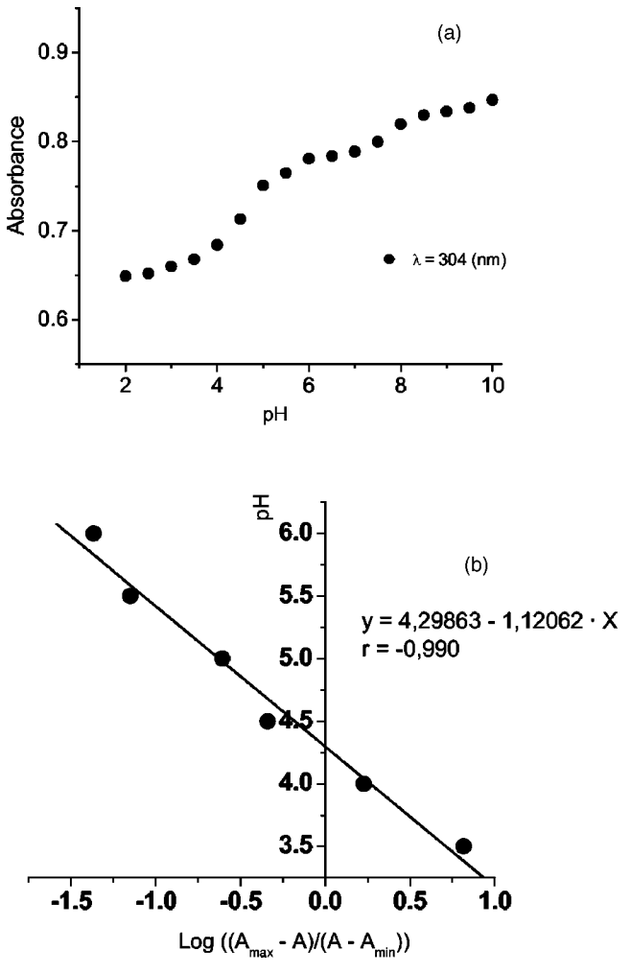

Figure 4. (a) Absorbance dependence of wave at λ = 304 nm of 4-MNImOH at different pH. (b) pK_a calculation according to linear regression method.

pK_a of the nitro-nitro protonated acid base equilibrium. To validate this point, we have calculated an equivalent pK_a value by using UV-spectrophotometry. UV spectra at different pH values reveal one pH-dependent absorption signal at 304 nm. The sensitivity of this signal to pH was used to determine the spectroscopic apparent pK_a (Fig. 4). The value of pK_a obtained for 4-MNImOH was 4.3, and it was calculated by the linear regression method. This value agrees with the break at 3.5 in the E_p *vs.* pH plot obtained with the DPP technique.

To elucidate the number of electrons transferred in the reduction process, we compared the limiting current of 4-MNImOH with the limiting current of equimolar solutions of the previously studied 4-nitroimidazole[27] under the same experimental conditions (pH $<$ 7). 4-MNImOH showed the same value of the limiting current measured by tast polarography, which indicates that the same four electrons are transferred (data not shown). We also carried out coulometric experiments at pH 7, finding that the number of electrons transferred was slightly lower than four, as shown in Table I (3.7 ± 0.1). However, coulometric experiments at pH $<$ 4 provided lower values, indicating that probably a part of the starting material is disappearing in a nonfaradaic process, as reported by Vianello *et al.*[26]

According to the well-known mechanism of 4-nitroimidazole,[27] we can assume that the observed irreversible peak at acid and neutral pH is due to the four-electron, four-proton reduction of the nitroimidazole group to yield the hydroxylamine derivative according to the following overall reaction

$$\text{R-NO}_2 + 4\overline{\text{e}} + 4\text{H}^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O} \qquad [2]$$

In the same way, the equations describing the two new signals at alkaline pH correspond to

$$\text{R-NO}_2 + \overline{\text{e}} \leftrightarrows \text{RNO}_2^{\bullet -} \qquad [3]$$

$$\text{RNO}_2^{\bullet -} + 3\overline{\text{e}} + 4\text{H}^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O} \qquad [4]$$

The observed splitting at alkaline pH by using DPP was also confirmed by cyclic voltammetry. Figure 5 displays CVs at three different pH conditions. In acid and neutral media, it is possible to observe only one irreversible signal due to the four-electron, four-proton reduction of the nitro compound to generate the hydroxylamine derivative according to Reaction 2. In alkaline conditions, we can observe the reversible couple, I_a/I_c, corresponding to the one-electron reduction of the 4-MNImOH according to Reaction 3 and the irreversible peak II_c corresponding to the further reduction of the nitro radical anion to the hydroxylamine derivative, according to Reaction 4. Furthermore, in the reverse sweep, an anodic peak,

Table I. DPP cathodic peak potentials ($E_{p,c}$) obtained from 4-MNImOH solutions in ethanol/Britton-Robinson buffer.

pH	$-E_{p,c}$ (mV)	n[a]
4	530	3.3 ± 0.1
7	676	3.7 ± 0.1

[a] The number of electrons transferred (n) was coulometrically obtained as the mean of five runs.

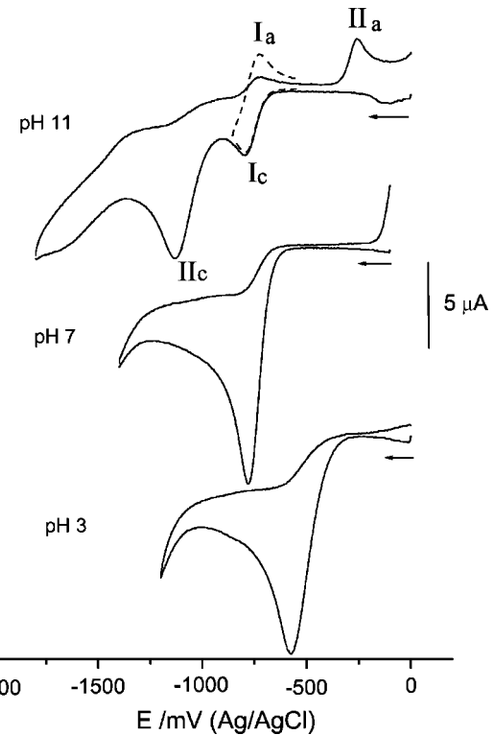

Figure 5. CVs of 1 mM 4-MNImOH in ethanol/Britton-Robinson buffer 0.1 M at different pH values. Sweep rate 1 V s^{-1}. Dashed line shows a short sweep with $RNO_2/RNO_2^{\bullet -}$ isolated couple. Arrow indicates the scan direction.

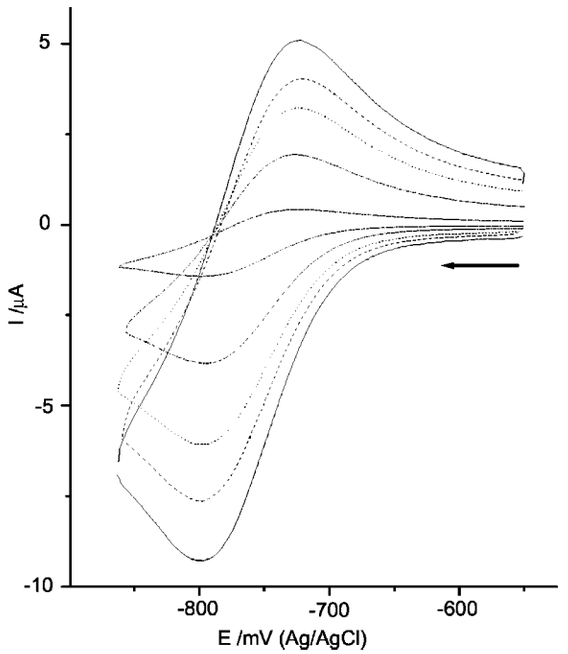

Figure 6. CVs showing the reversible couple due to the one-electron reduction of 4-MNImOH in nonaqueous medium at different sweep rates. Arrow indicates the scan direction.

II_a, at −256 mV was observed. This corresponds to the two-electron oxidation of the hydroxylamine derivative, which forms during the first negative-going sweep, to form the nitroso derivative

$$\text{R-NHOH} \rightarrow \text{R-NO} + 2\overline{e} + 4H^+ \qquad [5]$$

Adjusting the switching potential appropriately, we can study the nitro/nitro radical anion couple ($RNO_2/RNO_2^{\bullet -}$) in isolation (dashed line in Fig. 5). To complete the electrochemical characterization to study the properties of the nitro radical anion in the absence of protonation reactions, we also studied an aprotic medium. In a totally nonaqueous medium containing 100% DMF with 0.1 M TBAP, we obtained a perfectly isolated couple (Fig. 6) corresponding to the one-electron reduction of the nitroimidazole parent compound to produce the nitroimidazole radical anion derivative. The generation of the nitro radical anion derivative was also characterized by ESR. The radical was prepared *in situ* by controlled potential electrolysis at −900 mV *vs.* Ag/AgCl in DMF. The nitroimidazole free radical displays a well-resolved ESR spectrum (Fig. 7). The interpretation of the ESR spectra led us to determine the coupling constants for all

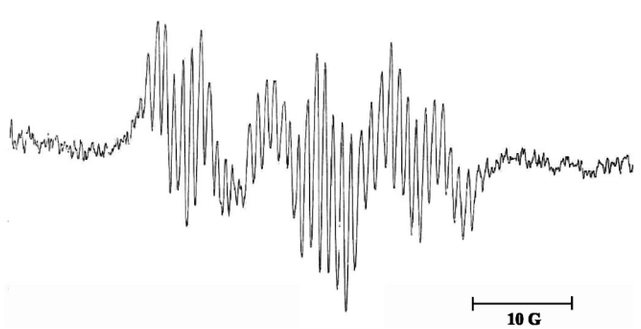

Figure 7. ESR experimental spectrum of radical anion of 4-MNImOH in DMF. Spectrometer conditions: microwave frequency 9.68 GHz, microwave power 20 mW, modulation amplitude 0.2 G, scan rate 1.25 G/s, time constant 0.5 s, number of scans: 15.

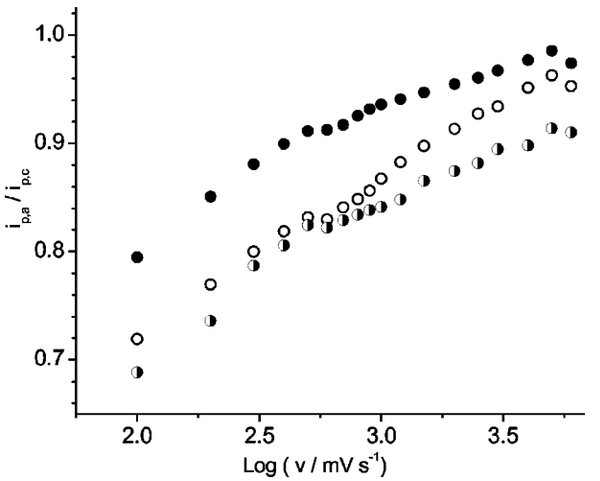

Figure 8. Current ratio dependence on sweep rates of $RNO_2/RNO_2^{\bullet -}$ couple from CVs of 1 mM 4-MNImOH in ethanol/Britton-Robinson buffer at different pH values.

magnetic nuclei. Thus, the obtained hyperfine constants were $a_N(NO_2)$ = 14.0 G, a_H = 4.5 G, a_N (ring) = 2.0 G, a_N (ring) = 1.2 G, and a_H (CH_3) = 0.4 G.

Using the preceding cyclic voltammetric response, we can study the kinetic stability of the nitro radical anion species in all the media conditions. In Fig. 8, we can observe the effect of different sweep rates on the current ratio, showing a similar general trend in all media, *i.e.*, the $i_{p,a}/i_{p,c}$ current ratio increases as the scan rate is increased. These results fulfill the requirements for an irreversible chemical reaction following a reversible charge-transfer step according to the classical Nicholson criteria,[30] but we also found that the current ratio was dependent on the 4-MNImOH concentration (data not shown), implying a second-order chemical step. To check what kind of second-order chemical reaction is involved, we tried both disproportionation and dimerization approaches. Using the theoretical approach of Olmstead *et al.* for disproportionation[31] and dimerization,[32] we calculated the second-order rate constant, k_2, obtaining different values depending on whether disproportionation or dimerization was assumed. To decide what type of mechanism adjusts better with the real mechanism, we used the simulation procedure described in a previous paper.[34] That procedure is based on the comparison between experimental and simulated curves, wherein the latter is simulated by both disproportionation and dimerization. In Fig. 9, we can observe a comparison of experimental and simulated CVs for 4-MNImOH considering either disproportionation or

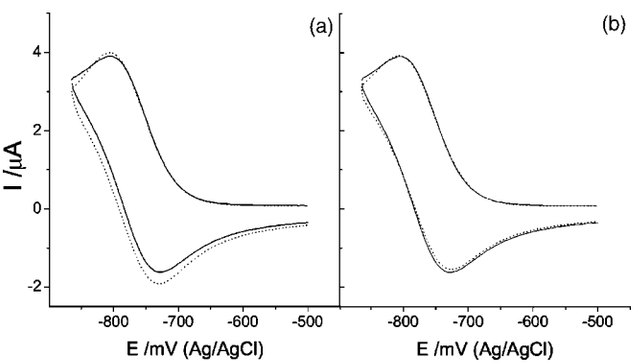

Figure 9. Comparison of experimental (solid line) and simulated (dashed line) CVs for 4-MNImOH in mixed medium considering (a) dimerization or (b) disproportionation as the chemical coupled reaction.

Table II. Disproportionation and dimerization rate constant and half-lifetime values for $RNO_2/RNO_2^{\bullet-}$ couple obtained for 4-MNlmOH in different media.

Medium	$10^{-3}k_2$ (M^{-1} s^{-1})	$t_{1/2}$ (s)
B/EtOH pH 10 [a]	5.97 ± 0.25 (disp)	0.17
B/EtOH pH 11	1.93 ± 0.24 (disp)	0.5
B/EtOH pH 12	2.23 ± 0.23 (disp)	0.45
DMF/citrate[b] pH 10	13.64 ± 2.13 (disp)	0.08
DMF	1.46 ± 0.11 (dim)	0.69

[a] 30/70: Ethanol/Britton-Robinson buffer (0.1 M).
[b] 60/40: DMF/citrate buffer (0.015 M), KCl (0.1 M).

dimerization in a mixed medium. From these results, one can see that in the case where disproportionation was considered, the fit between the simulation and experimental curves was clearly better. Moreover, we have obtained optimum correlation between experimental and simulated curves for disproportionation in mixed media (using ethanol or DMF as cosolvents) and dimerization in nonaqueous medium (100% DMF). Consequently, the change from mixed to nonaqueous medium produces a change in the decay mechanism of the radical anion passing from disproportionation in mixed medium to dimerization in a nonaqueous medium. This finding is in accord with previous results obtained for nitro radical anion produced from some nitrofuran derivatives.[34]

The obtained kinetic second-order rate constant, k_2, and the corresponding half-lifetime values for the nitro radical anion from 4-MNImOH in different media are shown in Table II. From these values, we can state that, as expected, the nitro radical anion was more stabilized in a totally nonaqueous medium, confirming that its optimal environment is the lipophilic one. However, when we compared different buffers at the same pH, we found different stabilities for the radical, showing that the proton activity is not the only factor promoting its decay. For the same buffer at strong alkaline conditions (pH 11-12), the results were statistically similar, showing the same stability. Furthermore, in Table III we compared the results obtained for 4-MNImOH with those obtained with 4-nitroimidazole. From these results, we can conclude that the new compound 4-MNImOH is more easily reducible than the 4-nitroimidazole, thus requiring less energy to produce the nitro radical anion. However, from the kinetic constants, we can conclude that the nitro radical anion from 4-MNImOH is less stable than the corresponding free radical from 4-nitroimidazole. Both of these results point to a product with enhanced capabilities to become a more useful bioactive compound than the parent molecule, 4-nitroimidazole. The new compound would be more suitable for enzymatic reduction and less toxic to the host than 4-nitroimidazole, meaning it would be a potential new bioactive 4-nitroimidazole compound.

Conclusions

The new synthesized compound, 4-MNImOH, was easily reducible in aqueous, mixed, and nonaqueous media, but only in mixed and nonaqueous media was it able to generate nitro radical anions capable of being detected in the time scale of the cyclic voltammetric technique. The nitro radical decayed according to a second-order chemical reaction, disproportionation in mixed media, and dimerization in a totally nonaqueous medium.

Table III. Comparison between cathodic potential peak values and nitro radical anion decay constants for one-electron reduction of 4-nitroimidazole and 4-MNImOH obtained in 30/70: ethanol/Britton-Robinson buffer (0.1 M), pH 10.

Compound	$-E_{p,c}$ (mV)	10^{-3} k_2 (M^{-1} s^{-1})
4-Nitroimidazole	850	2.82 ± 0.1
4-MNImOH	760	5.97 ± 0.2

According to the results, the new substituted 4-nitroimidazole derivative was more easily reducible than the nonsubstituted parent compound, and the corresponding nitro radical anion decays more rapidly than the nonsubstituted nitro radical. Both of these aspects are promising for finding more efficient bioactive compounds with sufficiently low reduction potentials in order to be enzymatically reduced and at the same time to have radicals not stable enough to produce damage in the host.

In addition, we believe that for compounds that act via free radicals, *e.g.*, nitroimidazole derivatives, the voltammetric determination of simple parameters, such as the reduction potential and stability constants, may be a very good option to carry out an *in vitro* first step screening to select a bioactive compound, avoiding more expensive and tedious procedures.

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References

1. D. I. Edwards, *J. Antimicrob. Chemother.*, **31**, 9 (1993).
2. P. Wardman, *Environ. Health Perspect.*, **64**, 309 (1985).
3. F. Riché, A. du Molinet, S. Sèpe, L. Riou, D. Fagret, and M. Vidal, *Bioorg. Med. Chem. Lett.*, **11**, 71 (2001).
4. D. I. Edwards, *Biochem. Pharmacol.*, **35**, 53 (1986).
5. W. Bone, N. Jones, G. Kamp, C. Yeung, and T. Cooper, *J. Reprod. Fertil.*, **118**, 127 (2000).
6. D. Church, H. Rabin, and E. Laishley, *J. Antimicrob. Chemother.*, **25**, 15 (1990).
7. C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitcheff, and R. Vaupre, *Arzneim.-Forsch.*, **16**, 23 (1966).
8. M. D'Auria, F. D'Onofrio, J. Suwinski, and K. Swierczek, *Tetrahedron*, **49**, 3899 (1993).
9. J. Suwinski, W. Szczepankiewicz, and M. Widel, *Arch. Pharm. (Weinheim)*, **325**, 317 (1992).
10. G. Elizondo, M. Gonsebatt, A. M. Salazar, I. Lares, P. Santiago, J. Herrera, E. Hong, and P. Ostrosky-Wegman, *Mutat Res.*, **370**, 75 (1996).
11. S. Gomez-Arroyo, S. Melchor-Castro, R. Villalobos-Pietrini, E. Melendez-Camargo, H. Salgado-Zamora, and M. Campos-Aldrete, *Toxicol. in Vitro*, **18**, 319 (2004).
12. S. Khabnadideh, Z. Rezaei, A. Khalafi, R. Bahrinajafi, R. Mohamadi, and A. Farrokhroz, *Bioorg. Med. Chem. Lett.*, **13**, 2863 (2003).
13. N. Boechat, A. Carvalho, E. Fernandez-Ferreira, R. Soares, A. Souza, D. Gibaldi, M. Bozza, and A. Pinto, *Cytobios*, **105**, 83 (2001).
14. T. Chu, S. Hu, B. Wei, Y. Wang, X. Liu, and X. Wang, *Bioorg. Med. Chem. Lett.*, **14**, 747 (2004).
15. D. Dumanovic, J. Volke, and V. Vajgand, *J. Pharm. Pharmacol.*, **18**, 507 (1966).
16. S. A. Ozcan, Z. Senturk, and I. Biryol, *Int. J. Pharm.*, **157**, 137 (1997).
17. A. Radi, S. El-Laban, and A.-G. El-Kourashy, *Electroanalysis*, **9**, 625 (1997).
18. S. A. Okzan, *Analusis*, **25**, 130 (1997).
19. S. Bollo, L. J. Núñez-Vergara, M. Bontá, G. Chauviere, J. Perie, and J. A. Squella, *Electroanalysis*, **13**, 936 (2001).
20. J. H. Tocher and D. I. Edwards, *Free Radical Res. Commun.*, **16**, 19 (1992).
21. D. Barety, B. Resibois, G. Vegoten, and Y. Moschetto, *J. Electroanal. Chem. Interfacial Electrochem.*, **162**, 335 (1984).
22. S. Bollo, L. J. Núñez-Vergara, M. Bontá, G. Chauviere, J. Perie, and J. A. Squella, *J. Electroanal. Chem.*, **511**, 46 (2001).
23. M. L. Arguelho, G. Silva, and N. Stradiotto, *J. Electrochem. Soc.*, **148**, D1 (2001).
24. P. C. Mandal, *J. Electroanal. Chem.*, **570**, 55 (2004).
25. D. Dumanovic, J. Jovanovic, D. Suznjevic, M. Erceg, and P. Zuman, *Electroanalysis*, **4**, 889 (1992).
26. S. Roffia, C. Gottardi, and E. Vianello, *J. Electroanal. Chem. Interfacial Electrochem.*, **142**, 263 (1982).
27. J. Carbajo, S. Bollo, L. J. Núñez-Vergara, A. Campero, and J. A. Squella, *J. Electroanal. Chem.*, **531**, 187 (2002).
28. A. G. Gonzalez, F. Pablos, and A. Asuero, *Talanta*, **39**, 91 (1992).
29. A. Asuero, M. A. Herrador, and A. G. Gonzalez, *Talanta*, **40**, 479 (1993).
30. R. S. Nicholson, *Anal. Chem.*, **36**, 1406 (1964).
31. M. L. Olmstead and R. S. Nicholson, *Anal. Chem.*, **41**, 862 (1969).
32. M. L. Olmstead, R. G. Hamilton, and R. S. Nicholson, *Anal. Chem.*, **41**, 260 (1969).
33. K. A. Connors, *Curso de análisis farmacéutico (Ensayo del medicamento)*, Editorial reverté, SA Barcelona, Spain, p. 145 (1980).
34. M. Bontá, G. Chauviere, J. Perie, L. J. Núñez-Vergara, and J. A. Squella, *Electrochim. Acta*, **47**, 4045 (2002).