Cyclic voltammetric study of the disproportionation reaction of the nitro radical anion from 4-nitroimidazole in protic media

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Abstract

We have chosen 4-nitroimidazole (4-NIm) as a prototype of nitroimidazolic compounds in order to carry out an exhaustive cyclic voltammetric study in protic media, 0.1 M Britton–Robinson buffer + 0.3 M KCl with ethanol as a co-solvent. The one electron reduction of 4-NIm in protic media at alkaline pH produces a stable nitro radical anion on the time scale of the cyclic voltammetric technique. The nitro radical anion decays according to a coupled chemical reaction and we have focused the study to follow this reaction using cyclic voltammetric methodology. The one electron reduction of the 4-NIm to form the nitro radical anion and the subsequent reaction of the radical obey an EC_2 mechanism that follows very well the cyclic voltammetry theory for the disproportionation reactions previously described by Olmstead and Nicholson (Anal. Chem. 41 (1969) 862). We have obtained the disproportionation rate constant k_2 which is strongly dependent on both pH and ethanol content according to the following regression equations: $\log k_2 = -0.932\mathrm{pH} + 12.771$ and $\log(10^{-3} k_2) = -1.998\log[\% \mathrm{EtOH}] + 3.873$. The results obtained from this study in protic media differ substantially from previous studies in aprotic media wherein the nitro radical anion was not stabilized.

1. Introduction

Nitroimidazoles are used extensively in the treatment of infectious illnesses in both human and non-human therapeutics [1,2]. The antimicrobial action of nitroimidazoles is produced inside pathogenic microorganisms wherein some reduced form of the nitro group interacts with the microbial DNA, thus causing damage in the microbial structure hindering its reproduction. Consequently, it is vital for the mechanism of action that the microorganisms have an enzymatic system capable of reducing the nitro group. The reduction of nitroimidazoles can be produced via two different routes according to the media conditions i.e. aerobic or anaerobic [3,4], but under both conditions the requirement of the first step of the reduction is the one-electron reduction to form the nitro radical anion:

$$RNO_2 + e^- \rightarrow RNO_2^{\cdot -} \tag{1}$$

Under anaerobic conditions, the nitro radical anion formed can undergo further reductions producing nitroso, hydroxylamine and amine derivatives:

$$RNO_2^{\cdot -} + e^- + 2H^+ \rightarrow RNO + H_2O \tag{2}$$

$$RNO + 2e^- + 2H^+ \rightarrow RNHOH \tag{3}$$

$$RNHOH + 2e^- + 2H^+ \rightarrow RNH_2 + H_2O \tag{4}$$

On the other hand, under aerobic conditions, the nitro radical anion formed would be re-oxidized to regenerate the initial nitro compound and to produce superoxide ($O_2^{\cdot -}$) provoking a so called 'futile cycle':

$$RNO_2^{\cdot -} + O_2 \rightarrow RNO_2 + O_2^{\cdot -} \tag{5}$$
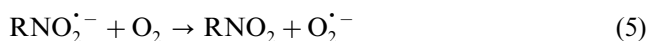
As can be seen the nitro radical anion plays a crucial and important role in the mechanism of the action of nitroimidazole compounds and consequently a knowledge of its behaviour is a permanent challenge because its radical chemistry is central to the use of nitroimidazoles in medicine.

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The prototropic properties and natural lifetimes of nitro radical anions have been studied mainly by using electron spin resonance (ESR) and pulse radiolysis techniques [5–7] but lately electrochemical techniques i.e. cyclic voltammetry, have also been shown to play an advantageous role in the study of nitro radical anions [8–14]. Under suitable conditions of the medium, the one electron reduction of nitroaromatic and nitroheterocyclic compounds produces very well resolved cyclic voltammograms due to the nitro/nitro radical anion couple ($RNO_2/RNO_2^{\cdot -}$). By using appropriately the wide versatility of the cyclic voltammetric technique, it is possible to study the feasibility of formation of nitro radical anions [8,9], their prototropic behavior [10], their natural lifetimes [11,12] and their reactivity with other molecules [13,14].

There are numerous studies related to electrochemical aspects of nitroimidazoles. Generally these are focused on the electroanalytical determination of some nitroimidazoles of importance in medicine such as metronidazole [15], ornidazole [16], secnidazole [17], tinidazole [18], and megazol [19]. Furthermore, the use of cyclic voltammetry in order to obtain the lifetimes of nitro radical anions from some nitroimidazoles such as misonidazole, metronidazole and megazol [20–22] has been also reported.

In this work we have centered our study on the nitro radical anion produced from a molecule such as 4-nitroimidazole (4-NIm) wherein the only substituent on the nitroimidazole moiety is the nitro group thus avoiding any possible distortion effect from other substituents. There has been only one previous report devoted to the electrochemical behaviour of 4-NIm in aprotic media wherein the autoprotonation of the nitro radical anion was shown but, consequently, that work did not involve a study of the nitro radical anion [23]. In the present study we report an exhaustive cyclic voltammetric study of the prototropic properties and the natural lifetime of the nitro radical anion obtained from 4-NIm. Specifically we have focused our study on the disproportionation reaction of the nitro radical anion from 4-NIm in a protic medium containing ethanol as a co-solvent. The influences of pH, ethanol content and 4-NIm concentrations on the disproportionation rate constant were investigated in depth.

2. Experimental

2.1. Reagents and solutions

4-NIm 97% pure was obtained from Aldrich Chem. Co. and was used without prior purification. All the other reagents employed were of analytical grade. Nitrogen gas was from Alphagas-Air liquide with maximum impurities of $H_2O < 3$ ppm; $O_2 < 2$ ppm; $C_nH_m < 0.05$ ppm.

All the voltammetric experiments were obtained after bubbling with N_2 for 10 min in the cell before each run. The temperature was kept constant at 25 ± 0.1 °C in all experiments.

Solutions for cyclic voltammetry were prepared starting from a 0.2 M stock solution of 4-NIm in DMF prepared daily. Final solutions (20 ml) 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 (Except in the 4-NIm concentration study).

Experiments for the pH study were made in Britton–Robinson buffer 0.1 M + 0.3 M KCl + EtOH (70//30). The pH was adjusted by addition of concentrated solutions of NaOH or HCl depending on the desired final pH. For the study of the dependence on the EtOH content, the EtOH% was varied appropriately in the above solution.

2.2. Apparatus

Voltammetric curves were recorded on an Autolab (Eco Chemie model PGSTAT 20) instrument attached to a PC computer with appropriate software (GPES 4.8 for Windows) for total control of the experiments and data acquisition and treatment. A static mercury drop electrode (SMDE) (Metrohm VA 663) with a drop area of 0.42 mm^2 was used as the working electrode and a glassy carbon rod as the counter electrode. All potentials were measured against Ag/AgCl/3 M KCl.

All pH measurements were carried out with a Crison model Basic 20 pH meter equipped with a glass electrode Crison 52-01 (resolution 0.01, precision ± 0.01). The standard solutions used for calibration were Crison 4.00 and 7.02 and Probus 10.00.

2.3. Methods

The return-to-forward peak current ratio I_{pa}/I_{pc}, for the reversible first electron transfer (the $R-NO_2/R-NO_2^{\cdot -}$ couple) was measured for each cyclic voltammogram, varying the scan rate from 0.05 up to 150 V s^{-1} according to the procedure described by Nicholson [24]. A reproducibility study involving ten independent runs at different sweep rates (0.5, 1 and 10 V s^{-1}) produced variation coefficients of 0.1 and 0.7% for potential peak and current ratio measurements, respectively.

Using the theoretical approach of Olmstead and Nicholson [25], 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, 4-NIm concentration and scan rate. A plot of ω versus 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 decomposition of $RNO_2^{\bullet -}$, c_o is the nitrocompound concentration and $\tau = (E_\lambda - E_{1/2})/v$. Consequently we can obtain the second order rate constant for the decomposition of the nitro radical anion from the slope of the straight line ω versus τ.

3. Results and discussion

4-NIm can be electrochemically reduced over the whole pH range at the mercury electrode in protic media (0.1 M Britton–Robinson buffer + 0.3 M KCl with ethanol as a co-solvent), but its reduction is strongly affected by pH changes. Consequently, the cyclic voltammetric response of the 4-NIm reduction is also pH-dependent. In fact, at pH values < 7 the cyclic voltammetric response corresponds to only one irreversible reduction peak without any oxidation peak appearing in the corresponding positive sweep. Obviously this reduction peak is due to the well-known [26] four-electron four-proton reduction of the nitro group to produce the corresponding hydroxylamine derivative:

$$RNO_2 + 4H^+ + 4e \rightarrow RNHOH + H_2O \qquad (6)$$

In Fig. 1 we can observe the behaviour of this voltammetric reduction peak in acidic media. At pH values > 7 the cyclic voltammetric response is rather different but follows the well-known [27] general pattern due to nitrocompound in aprotic media or in the presence of
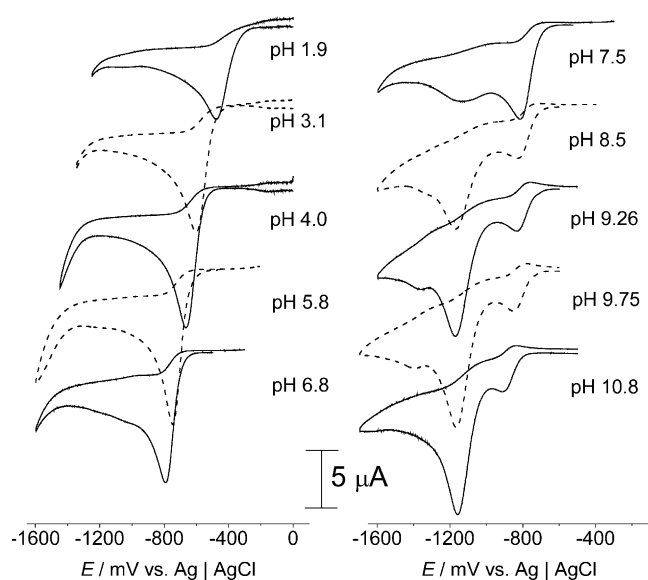
inhibitors. In fact the single signal observed in acidic media was divided into two different new signals according to the pH increase (Fig. 1). Consequently, at alkaline pH, the equations describing the new two signals correspond to:

$$RNO_2 + e^- \rightleftarrows RNO_2^{\bullet -} \qquad (7)$$
$$RNO_2^{\bullet -} + 3e^- + 4H^+ \rightarrow RNHOH \qquad (8)$$

In Fig. 2 we have summarized the pH effect on both cathodic peak current and negative peak potentials of both signals. As the aim of this paper is the cyclic voltammetric study of the nitro radical anion $(RNO_2^{\bullet -})$ produced by the one electron reduction of 4-NIm we have centered our study on the corresponding first reversible couple obtained at pH values > 7.

By working at sufficiently alkaline pH and adjusting the switching potential appropriately we can study the nitro/nitro radical anion couple $(RNO_2/RNO_2^{\bullet -})$ in isolation. In Fig. 3, a very good resolved cyclic voltammograms obtained after two consecutives sweeps is shown. Furthermore, we have obtained a ΔE_p value of approximately 60 mV (Table 1) confirming the one electron character of the couple.

With the aim of obtaining the pH-dependence of the $RNO_2/RNO_2^{\bullet -}$ couple we have carried out a very

Fig. 1. Cyclic voltammograms of 1 mM 4-NIm in protic media (0.1 M Britton–Robinson buffer + 0.3 M KCl + EtOH (70//30)) at different pH values. Sweep rate 1 V s^{-1}.
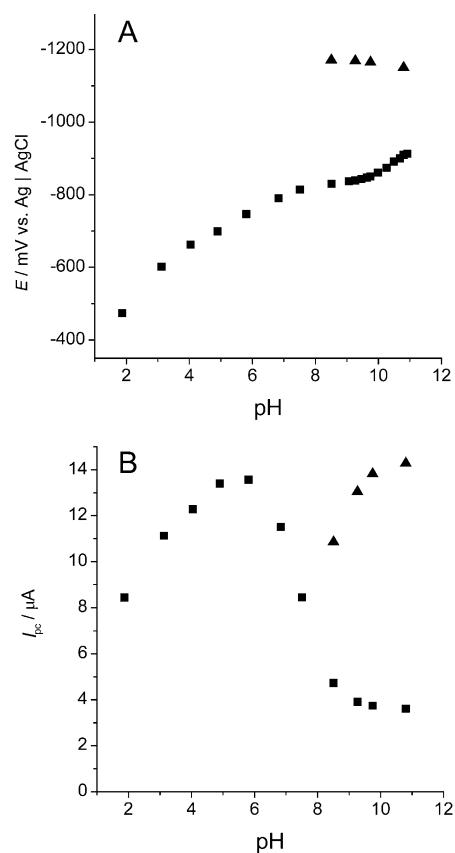

Fig. 2. Peak potential (A) and peak current (B) dependence with pH for 1 mM 4-NIm in protic media. ■, first peak; ▲, second peak. Other conditions as in Fig. 1.
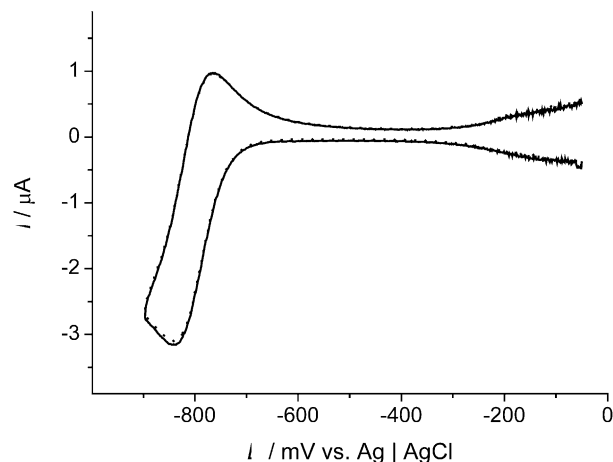
Fig. 3. Two consecutives cyclic voltammograms due to the $RNO_2/RNO_2^{\cdot-}$ couple from 1 mM 4-NIm in protic media. pH 9.10, $v = 1.0$ V s^{-1}. Other conditions as in Fig. 1.

Table 1
ΔE_p values of the $RNO_2/RNO_2^{\cdot-}$ couple obtained from 1 mM 4-NIm cyclic voltammograms in protic media, 30% EtOH, pH 9.15 at different sweep rates

v/V s^{-1}	ΔE_p/V
1.0	0.070
2.0	0.067
3.0	0.062
5.0	0.061
7.0	0.059

Fig. 4. Half-wave potential (A) and peak current (B) dependence on pH of the $RNO_2/RNO_2^{\cdot-}$ couple from cyclic voltammograms of 1 mM 4-NIm in protic media at different pH values. ▲, I_{pc}; ■, I_{pa}. Sweep rate 1 V s^{-1}. Other conditions as in Fig. 1.

Fig. 5. Current ratio dependence on sweep rates of the $RNO_2/RNO_2^{\cdot-}$ couple from cyclic voltammograms of 1 mM 4-NIm in protic media at different pH values. ▽, 9.45; ▼, 9.19; ○, 8.95; ●, 8.70, □, 8.44; ■, 8.19. Other conditions as in Fig. 1.

exhaustive study in a rather narrow zone ($8 <$ pH < 10). The change of pH clearly affects the cyclic voltammograms of the $RNO_2/RNO_2^{\cdot-}$ couple. Specifically, we observe two main changes when the pH increases; first, a shift of the wave to more negative values and second, a change in the peak current intensities, i.e. a decrease in the cathodic peak current and an increase in the anodic peak current. These changes are summarized in Fig. 4. Obviously, this observed pH-influence indicates that the one electron reduction of 4-NIm is not explained well by only one equation such as the above Eq. (7). Probably a chemical reaction involving protons should be coupled with the one-electron electrochemical reaction. This assumption is also supported by the observed behaviour of the couple when the sweep rate is varied. According to the Nicholson and Shain criteria [28] we can affirm that the process corresponds to an electron transfer with a coupled chemical reaction i.e. an EC mechanism. Consequently an increase of the current ratio (I_{pa}/I_{pc}) with an increase of the sweep rate (v) was observed at all the pH values studied, as is shown in Fig. 5. In order to investigate the order of the coupled chemical reaction we have tested cyclic voltammograms at different 4-NIm concentrations. According to the dependence obtained between I_{pa}/I_{pc} and the 4-NIm concentration (Fig. 6a)
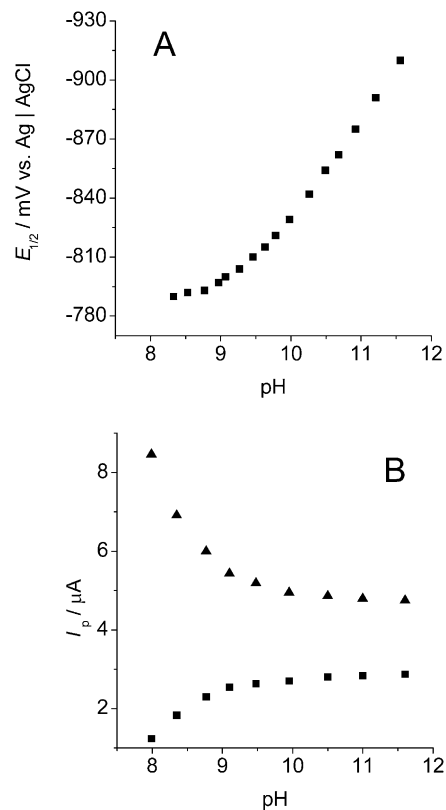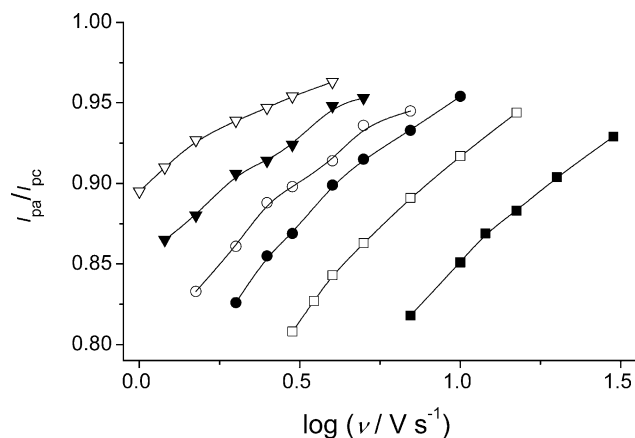
we can affirm that the order of the coupled reaction was different from order 1 and from the slope of the plot of the half-wave potential ($E_{1/2}$) versus the natural logarithm of 4NIm concentration (Fig. 6b) [29] we obtained an order of 2 for the coupled chemical reaction. Consequently from this conclusive experimental evi-
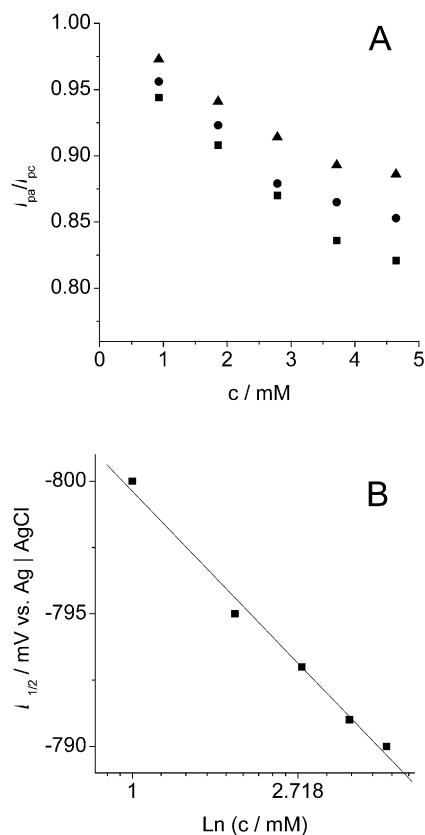
Fig. 6. (A) Current ratio dependence on the 4-NIm concentration at different sweep rates: ▲, 7.0; ●, 5.0; ■, 4.0 V s^{-1} and (B) half-wave potential dependence with the natural logarithm of 4-NIm concentration in protic media. pH 9.10. Other conditions as in Fig. 1.

dence and the well-known fact (7) that nitro radical anions can suffer a disproportionation reaction we can affirm that the second order coupled chemical reaction corresponds to a disproportionation reaction, producing the following EC$_2$ mechanism:

$$RNO_2 + e^- \rightleftarrows RNO_2^{\cdot -} \quad (7a)$$

$$2RNO_2^{\cdot -} + 2H^+ \rightarrow RNO_2 + RNO + H_2O \quad (9)$$
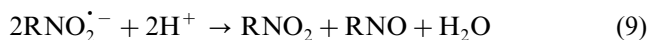
In order to confirm the proposed mechanism we applied the cyclic voltammetry theory for the disproportionation reaction described by Olmstead and Nicholson [25]. In fact we obtained straight lines in the plots of the kinetic parameters ω versus τ in perfect accordance with that anticipated by the theory (Fig. 7A). Furthermore, according to the theory, for values $a\tau = 4$, it should be noted that $\omega = k_2 c^o \tau$; consequently, we can obtain the disproportionation rate constant (k_2) from the slope of the straight lines between ω and τ. We have calculated the k_2 values and the corresponding half-life times ($t_{1/2}$) for the nitro radical anion from 4-NIm at different pH values (Table 2). As can be seen from these results the stability of the nitro radical anion is related directly to the proton concentration of the medium i.e. a lower proton concentration implies higher stability. Moreover,

the logarithm of the disproportionation rate constant follows a linear dependence with pH (Fig. 7B). From this behaviour it is possible to obtain the following regression curve: $\log k_2 = -0.932\text{pH} + 12.771$ ($r^2 = 0.9949$); which permits the k_2 value at different pH values to be calculated by extrapolation. By using the above equation we have obtained a k_2 value of 7.48×10^5 M^{-1} s^{-1} and a $t_{1/2}$ of 1.33×10^{-3} s at pH 7.4. Obviously these values are in accord with the experimental fact that the nitro radical anion cannot be observed on the time scale of the cyclic voltammetric experiment at pH 7.4 in protic media.

In order to study the dependence of 4-NIm concentration on the stability of the nitro radical anion we have selected pH 9.10. As was shown above (Fig. 6) both the I_{pa}/I_{pc} and the $E_{1/2}$ fulfilled the requirements for a second order chemical reaction. In order to examine the character of the limiting current we have studied the behaviour of the cathodic peak current, I_{pc}, with the sweep rate at different 4-NIm concentrations. The logarithmic plot of these parameters is shown in Fig. 8. As can be observed we have obtained a linear dependence with a slope of 0.5 showing that the one-electron reduction of 4-NIm corresponds to a diffusion controlled process without adsorption interference.

Furthermore, in a similar way as shown above, we have used the theory described by Olmstead and Nicholson [25] to calculate the disproportionation rate constant, k_2, at different 4-NIm concentrations. In Fig. 9 we can observe the experimentally obtained linear behaviour of the kinetic parameters ω versus τ at different 4-NIm concentrations. From the slopes of these lines we have obtained both k_2 and $t_{1/2}$ at different 4-NIm concentrations (Table 3). From these results we can observe that k_2 remains practically constant at different 4-NIm concentrations as is expected for a rate constant. However, as is expected for a second order chemical reaction, the $t_{1/2}$ of the nitro radical anion is dependent of the 4-NIm concentration. At lower concentrations of 4-NIm the nitro radical anion is more stable. As in-vivo concentrations would be relatively low, probably the in-vivo nitro radical anion stability would be high which could have dangerous consequences.

In order to study the influence of the media in the stability of the nitro radical anion we have varied the ethanol content in the solutions studied. The appearance of the cyclic voltammogram of the one-electron redox couple was affected by the quantity of ethanol in the medium. This change can be shown clearly from some cyclic voltammetric parameters such as $E_{1/2}$ and I_{pa}/I_{pc} as is shown in Fig. 10. From these results it is clear that an increase in the ethanol content produces both a shift in $E_{1/2}$ and an increase in I_{pa}/I_{pc}; however, different quantities of ethanol did not affect the mechanism of the disproportionation reaction as is deduced from the
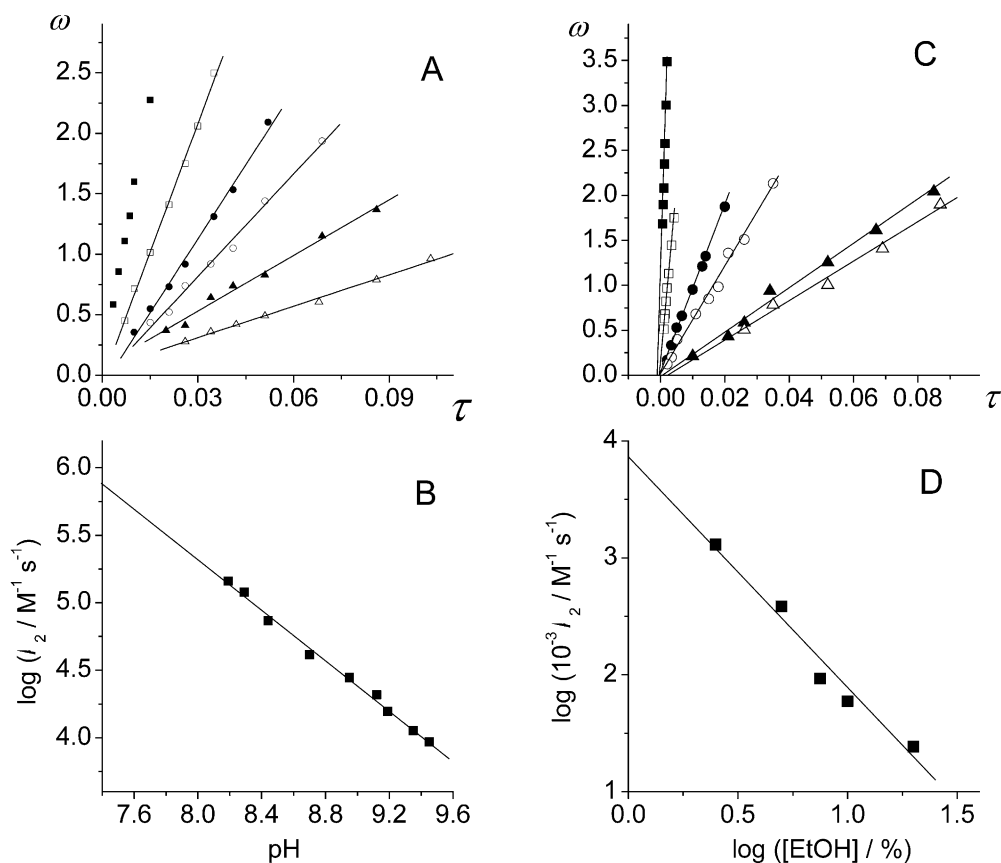
Fig. 7. (A–C) Plots of the kinetic parameter, ω, with the time constant, τ, for 1 mM 4-NIm and 30% EtOH at different pHs: ■, 8.19; □, 8.44; ●, 8.70; ○, 8.95; ▲, 9.19; △, 9.45 (plot A) and at different ethanol percentages, pH 9.10: ■, 2.5; □, 5.0; ●, 7.5; ○, 10.0; ▲, 20.0, △, 30.0% (plot C). (B–D) Disproportionation rate constant dependence on pH for 30% EtOH (plot B) and for an ethanol content at pH 9.10 (plot D) for 1 mM 4-NIm in protic media. Other conditions as in Fig. 1.

Table 2
Disproportionation rate constant and half life times values for $RNO_2/RNO_2^{\cdot-}$ couple obtained from 1 mM 4-NIm in protic media, 30% EtOH at different pHs

pH	$10^{-3}\,k_2/l\,mol^{-1}\,s^{-1}$	$10^2\,t_{1/2}/s$
8.19	145	0.68
8.29	120	0.83
8.44	73.7	1.5
8.70	41.2	2.6
8.95	27.9	3.6
9.12	20.7	5.2
9.19	15.8	6.3
9.35	11.3	8.8
9.45	9.31	12

Fig. 8. Log–log dependence of the cathodic peak current of the $RNO_2/RNO_2^{\cdot-}$ couple on sweep rate at different 4-NIm concentrations in protic media, pH 9.10. ▲, 4.65; ○, 3.72; ●, 2.79; □, 1.86; ■, 0.93 M. Other conditions as in Fig. 1.

linear behaviour obtained for ω versus τ shown in Fig. 7C. From the slopes of these lines we have obtained both k_2 and $t_{1/2}$ at different ethanol contents. As can be seen from the results in Table 4, both the disproportionation rate constant, k_2, and the half-life time, $t_{1/2}$, have a strong dependence on the ethanol content in the medium. At lower ethanol concentrations (< 10%) the variation of k_2 and $t_{1/2}$ are very pronounced but at
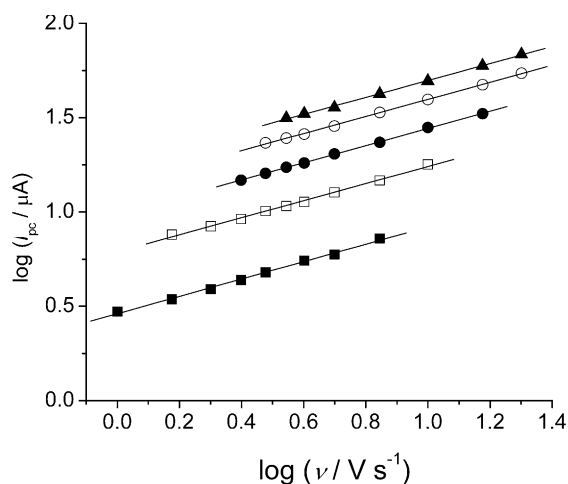
ethanol concentrations > 20%, the k_2 and $t_{1/2}$ values remain practically constant. Moreover, the logarithm of the disproportionation rate constant follows a linear dependence with the ethanol content in the medium
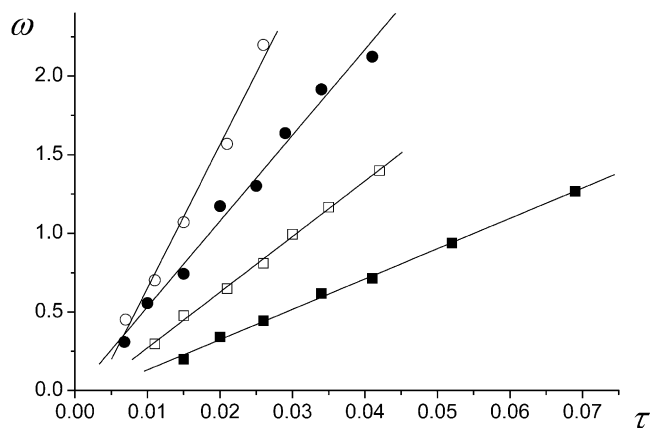
Fig. 9. Plot of the kinetic parameter, ω, vs. the time constant, τ, for different concentrations of 4-NIm in protic media, pH 9.10. ■, 0.93; □, 1.86; ●, 2.79; ○, 4.65 M. Other conditions as in Fig. 1.

Table 3
Disproportionation rate constant and half life times values for $RNO_2/RNO_2^{\cdot-}$ couple obtained from different concentrations of 4-NIm in protic media, 30% EtOH, pH 9.15

[4-NIm]/mM	$10^{-3} k_2$/l mol^{-1} s^{-1}	$10^2 t_{1/2}$/s
0.93	20.7	5.2
1.86	18.9	2.9
2.79	19.7	1.8
3.72	21.0	1.3
4.65	20.0	1.1

(Fig. 7D). From this behaviour, it is possible to obtain the following regression equation: $\log 10^{-3} k_2 = -1.998 \log[\% \text{ EtOH}] + 3.873$ ($r = 0.998$), for [% EtOH] $\leq$ 20%. Then, by using this linear relationship we obtained an extrapolated k_2 value of 7.46×10^6 M^{-1} s^{-1} and a $t_{1/2}$ value of 1.33×10^{-4} s at 1% EtOH concentration. Obviously from these results we can conclude that ethanol in the medium stabilizes the nitro radical anion strongly.
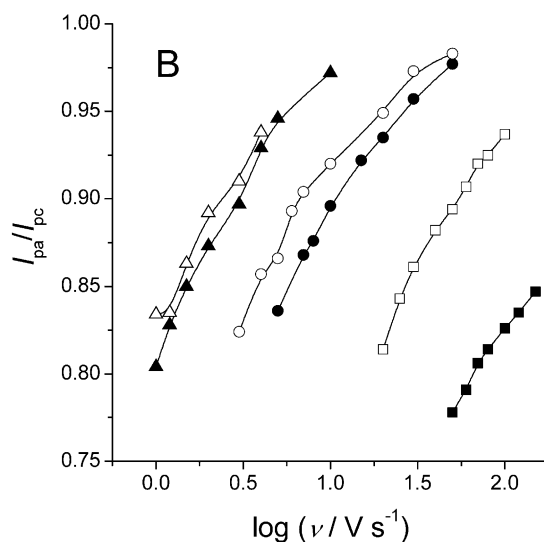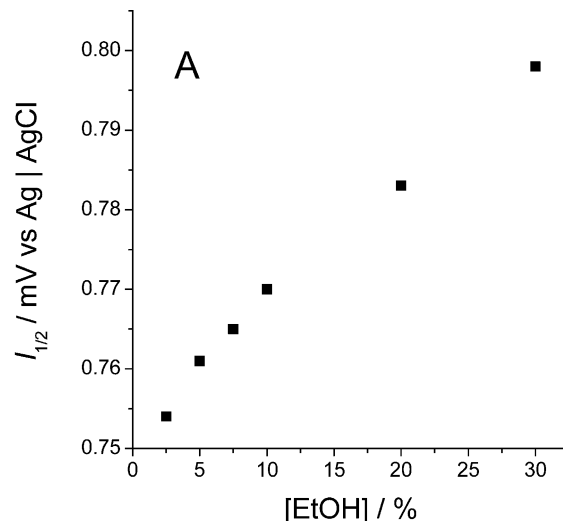

Fig. 10. (A) Half-wave potential dependence on the ethanol concentration and (B) peak current ratio dependence with the sweep rate at different ethanol concentrations: ■, 2.5; □, 5; ●, 7.5; ○, 10; ▲, 20; and △, 30%, for the $RNO_2/RNO_2^{\cdot-}$ couple from 1 mM 4NIm in protic medium at pH 9.10. Other conditions as in Fig. 1.

4. Conclusion

The results of experimental work presented above as well as those of previous workers can be summarized in the following points.

4-NIm was easily reduced in a protic media but by a totally different mechanism to that previously reported for this compound in aprotic (DMF, AN) media by Roffia et al. [23]. The cyclic voltammogram in aprotic media displayed two reduction peaks. The first peak was irreversible up to a sweep rate of 250 V s^{-1} while the second one appeared to be reversible even at low sweep rates. The first irreversible peak was attributed to the following overall electron reaction:

Table 4
Disproportionation rate constant and half life times values for $RNO_2/RNO_2^{\cdot-}$ couple obtained from 1 mM 4-NIm in protic media, pH 9.15, at different EtOH percentages

EtOH/%	$10^{-3} k_2$/l mol^{-1} s^{-1}	$10^2 t_{1/2}$/s
2.5	1300	0.08
5.0	385	0.26
7.5	93.2	1.1
10.0	59.4	1.7
20.0	24.5	4.1
30.0	21.9	4.6

$$5 HRNO_2 + 4e^- \rightarrow HRNHOH + 4RNO_2^- + H_2O \quad (10)$$

and the second reversible peak was attributed to the following reduction reaction which generates a radical nitro dianion:

$$RNO_2^- + e^- \rightleftarrows RNO_2^{2-\bullet} \quad (11)$$

On the other hand our results in protic media reveal cyclic voltammograms displaying: (a) at pH < 7; one irreversible reduction peak described by Eq. (6); and (b) at pH > 7; one reversible and one irreversible peak described by Eqs. (7) and (8).

According to Vianello's paper in aprotic media the $RNO_2/RNO_2^{\bullet-}$ couple cannot be recorded due to a rapid decay of the $RNO_2^{\bullet-}$ because of a fast protonation reaction by the starting nitroimidazole (a father–son type of reaction) with the formation of the conjugate base $RNO_2^{\bullet-}$ and of the neutral radical $RNO_2H^\bullet$. Paradoxically our results demonstrate that the $RNO_2/RNO_2^{\bullet-}$ couple can be very well stabilized in protic media but at alkaline pH, due to the autoprotonation, the couple is not favoured in this medium.

On the other hand, 4-NIm is a rare case wherein its nitro radical anion is sufficiently stabilized in protic media to be detected on the time scale of the cyclic voltammetric technique. Usually the detection of this radical requires the presence of inhibitors or aprotic solvent, besides alkaline pH. Furthermore, this nitro radical anion, generated from 4-NIm, is a very good example of the accomplishment of the theory for the disproportionation reactions previously described [29]. In spite of cyclic voltammetry results proving to be a very good tool in the study of nitro radical anions, care should be taken in the use of a co-solvent because it affects the stability of the radicals strongly.

Acknowledgements

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