Micellar Effects on the Reduction of 4-Nitroimidazole Derivative: Detection and Quantification of the Nitroradical Anion

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Abstract

The reductive electrochemistry of 1-methyl-4-nitro-2-hydroxymethylimidazole, a 4-nitroimidazole derivative, was examined in the presence of surfactants anionic as sodium dodecyl sulfate (SDS), nonionic, Triton-X, Cationic, Hyamine and Cetyl Trimethyl Ammonium Bromide (CTAB). The reductive mechanism of the nitroimidazole derivative was found to be dependent of both, nature and concentration of the surfactants.

By using appropriately the wide versatility of the cyclic voltammetric technique it was possible to study the generation of the nitroradical anion and their stability in different micellar media. The experimental scheme involved the measurement of the current ratio, $i_{p,a}/i_{p,c}$, from the cyclic voltammograms and its interpolation into theoretical working curves to determine the ω parameter. A plot of ω versus τ resulted in a linear relation described by $\omega = k_2 \, C \, \tau$ where k_2 is the second order rate constant for the decomposition of the nitroradical anion.

Cyclic voltammetric experiments demonstrated that the reduction of the 4-nitroimidazole derivative in presence of cationic micelles generated a sufficiently well stabilized nitroradical anion in aqueous medium at pH 7.4.

The aim of this study is to investigate the electrochemistry of 4-MNImOH in a micellar medium with the objective to obtain a better approach to the in-vivo conditions and to improve the detection and quantification of the nitroradical anion species.

Keywords: 4-Nitroimidazole, Micelles, Cyclic voltammetry, Nitroradical anion

1. Introduction

Nitroimidazoles have been the source of many investigations because of their properties as antibiotics, radiosensitizers and antiprotozoans. Its use as radiosensitizers takes advantage of its cytotoxicity in hypoxic mammalian cells what allows to increase sensitivity to the radiation of tumors [1–4]. Its antimicrobial characteristic takes advantage of its selective toxicity towards anaerobic microorganisms and allows that these compounds are used extensively in the treatment of infectious diseases in human and animal therapeutic [5–10]. Furthermore, is very well-known that nitroradical anion is a key intermediate in the biological activity of nitroimidazole derivatives [11]. Consequently, the understanding of the nitroradical behavior is a permanent challenge for this type of compounds and the electrochemistry can play an important role in its study.

The unique properties of surfactants, in particular their adsorption at interfaces and ability to form a variety of organized assemblies in solutions has been exploited in several fields of chemistry such as the electrochemistry. Saveant et al. [12] have reported a remarkable stabilization of the electrogenerated anion radical of phthalonitrile in the presence of cationic micelles and suggested that the observed 250-fold decrease in the rate of protonation of the anion radical was due to its association with the positively charged micelles. On the other hand, McIntire et al. [13], examined the electrochemistry of nitrobenzene in anionic, cationic and nonionic micelles. Results indicate that only anionic micelles stabilized the nitrobenzene anion radical due to a strong surface interaction.

In the scope of our current investigations to find new pharmacological important compounds that use the nitroradical anion as the active specie, we synthesized 1-methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH) a new 4-nitroimidazole derivative 1, 2- substituted. In a previous study [14], we studied the nitroradical anion formation in aqueous, mixed and nonaqueous media, but the nitroradical anion was detected only in mixed and nonaqueous media.

Taking into account that the micellar environment is considered to be a primitive, although simple, model for biological membranes [15], the present cyclic voltammetric study would be a very useful tool to quantify the stability and reactivity of the nitroradical anion in conditions that resembling the cell environmental.

2. Experimental

2.1. Reagents and Solutions

1-Methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH) (Fig. 1) was synthesized and characterized in our laboratory according to the procedure previously described [15]. The surfactants employed: sodium dodecyl sulfate (SDS) anionic; Triton-X, nonionic; Hyamine, Cationic and Cetyl Trimethyl Ammonium Bromide (CTAB) cationic were obtained from Sigma-Aldrich. All the other reagents employed were of analytical grade and were used without prior purification. Nitrogen gas was obtained from AGA Chile S. A. with maximum impurities of $H_2O < 3$ ppm; $O_2 < 2$ ppm; $C_nH_m < 0.5$ ppm.

All the voltammetric experiments were obtained after bubbling with N_2 for 10 min in the cell before each run. Temperature was kept constant at $25 \pm 0.1\,°C$ in all experiments.

0.1 M Britton–Robinson buffer was prepared dissolving 6.18 g of boric acid, 5.7 mL of glacial acetic acid, 6.7 mL of phosphoric acid and completing up to 1000 mL with water, and then the desired pH was adjusted with concentrate solutions of NaOH. The water used was purified with Milli-Q Ultra-Pure Water System.

Solutions containing surfactants were prepared with concentrations higher than the critical micelle concentration (CMC) in 0.1 M Britton–Robinson buffer.

4-MNImOH stock drug solution was prepared dissolving 7.85 mg and diluted up to 5 mL with 0.1 M Britton–Robinson buffer, to obtain a final concentration of 0.01 M.

Work solution was prepared taken an aliquot of the stock solution and diluted to 10 mL with 0.1 M Britton–Robinson buffer with and without surfactants.

2.2. Apparatus and Methods

Voltammetric curves were recorded on an Electrochemical Analyzer BAS CV-50W (Bioanalytical System) attached to a PC computer with appropriate software (BAS 100 W 2.3 for Windows) for total control of the experiments and data acquisition and treatment. A static mercury drop electrode with mercury drop area of 0.43 mm² (SMDE) (BASi EF-
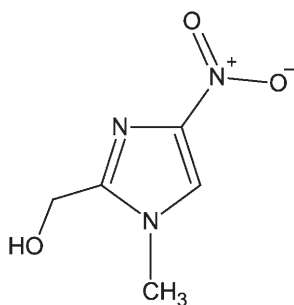
1400) for CV and a controlled growth mercury electrode (CGME) for polarography was used as the working electrode and a platinum wire (BASi MW-1032) as the counter electrode. All the potential measurements were carried out using an Ag|AgCl|NaCl (3 M) (BASi MF-2052) as the reference electrode.

For the kinetic analysis the return-to-forward peak current ratio $i_{p,a}/i_{p,c}$ for the one-electron couple ($RNO_2/RNO_2^{•-}$) was measured for each cyclic voltammogram followed by a data treatment procedure detailed previously [14].

3. Results and Discussion

4-MNImOH was electrochemically reduced in aqueous medium containing 0.1 M Britton–Robinson buffer when it is submitted to differential pulse polarography (DPP) and Tast polarography (TP) between pH 2–12. As can be observed in Figure 2, only one cathodic peak in all the pH range is observed. The peak has a cathodic peak potential, $E_{p,c}$, of −470 mV at pH 7 but the peak is strongly pH-dependent. In order to study the pH influence on the nitroreduction we have evaluated the behavior of the peak potential, (E_p) obtained by DPP, and the limiting current (I_{lim}) obtained by TP, at different pH between 2 and 12. In the insert of Figure 2, the E_p vs. pH plot shows that the peak potential is pH-dependent shifting to more negative potentials when pH increased up to pH 9.5, but upon pH 9.5 the peak potential remained constant. The limiting currents
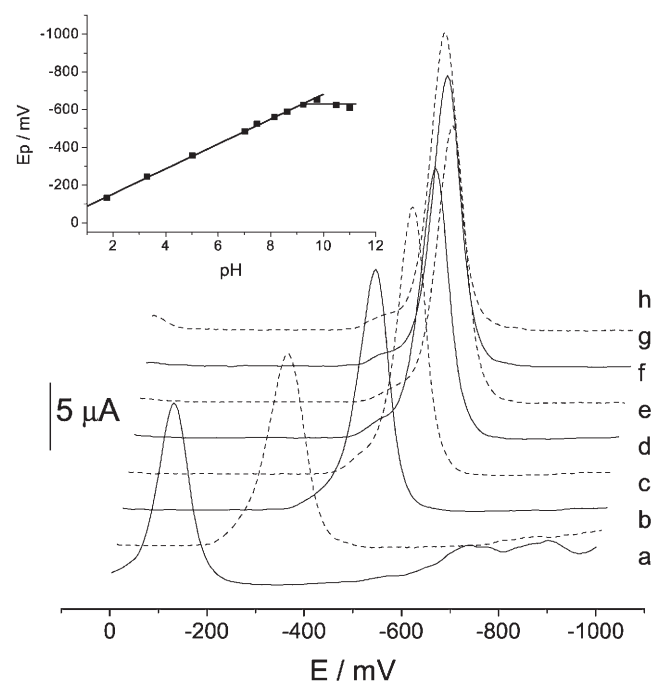

Fig. 2. Differential pulse polarograms of 0.1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer at different pHs between 2–11. Insert: The corresponding peak potential dependence with pH: 1.8 (a), 5.0 (b), 7.5 (c), 8.6 (d), 9.2 (e), 9.7 (f), 10.5 (g), 11.0 (h).

Fig. 1. Molecular structure of 1-methyl-4-nitro-2-hydroxymethylimidazole (4-MNImOH).

remain practically pH independent between pH 2–9.5, according to a diffusion controlled process (Fig. 3). As can be proved in a previous paper [14] the reduction of 4-MNImOH produces the hydroxylamine derivative according to the well-known Equation 1:

$$RNO_2 + 4e^- + 4\,H^+ \rightarrow RNHOH + H_2O \qquad (1)$$

The cyclic voltammogram of 4-MNImOH in aqueous medium containing 0.1 M Britton–Robinson buffer also shows a single peak with not any signal in the anodic sweep, which indicates that this peak corresponds to an irreversible process.

As can be observed from the above experiments it was not possible to stabilize the nitroradical anion in the aqueous medium. Considering that micelles could produce changes in the stability of the nitroradical anion we have added different types of surfactants to the aqueous solution to study the reduction of the nitroderivative.

The cathodic behavior of 4-MNImOH in aqueous medium was altered when surfactants were added to the solutions. We have tested anionic (SDS), nonionic (Triton X-100) and cationic (Hyamine and CTAB) surfactants but the observed modifications were dependent of the type of used surfactants. In the case of cationic surfactants the changes were more significant. The DPP of 4-MNImOH in CTAB and Hyamine micelles at different pHs are reflected in Figures 4A and B, respectively. From these figures is clear that the polarographic behavior was different from the situation without micelles (Fig. 2). Specifically the morphology of the polarographic peak changed i.e., the only peak observed in all the solutions without micelles is changed by two peaks in the new situation with micelles. In fact the change in the morphology of the polarographic response represents a change in the mechanism of the electrodic process changing from one overall step described in Equation 1 to two steps mechanism described by the well-known [15] following Equations 2 and 3:

$$RNO_2 + e^- \rightarrow RNO_2^{\cdot-} \qquad (2)$$

$$RNO_2^{\cdot-} + 3\,H^+ + 4\,H^+ \rightarrow RNHOH + H_2O \qquad (3)$$

Also, both potential peaks and current peaks for the reduction of 4-MNImOH changed with the addition of surfactants in all the pH range. The change of the potential peak (ΔE_p) for the nitroreduction resulting of the addition of CTAB and Hyamine in all the pH scale is shown in Figures 5A, B. As is noted from this Figure the ΔE_p is strongly pH-dependent exhibiting decreasing values when pH increased. The E_p values changed from about 400 mV at pH 2 to about 100 mV at pH > 10 meaning that in any pH condition the reduction of the nitrocompound in the micelles environment is more difficult than the situation without micelles. The E_p shift is consistent with the association of 4-MNImOH to the micellar aggregates showing that the NO_2 group is inserted into the CTAB or Hyamine micelles and hence more difficult electron-transfer associated with the reduction of the nitrogroup would be expected.

In the case of the anionic (SDS) and nonionic (Triton-X) surfactants the situation was totally different because the
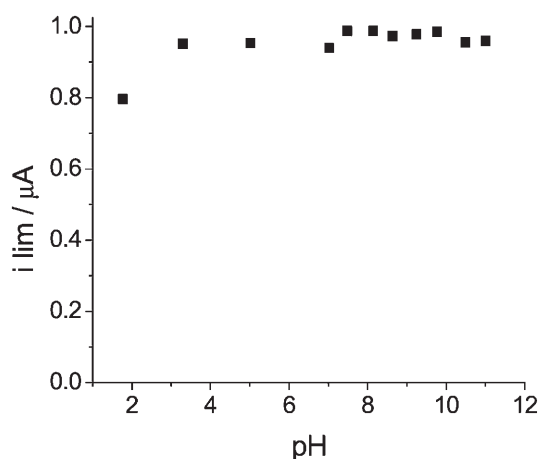

Fig. 3. Limiting current versus pH dependence obtained from Tast polarograms of 0.1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer at different pHs between 2 and 11.
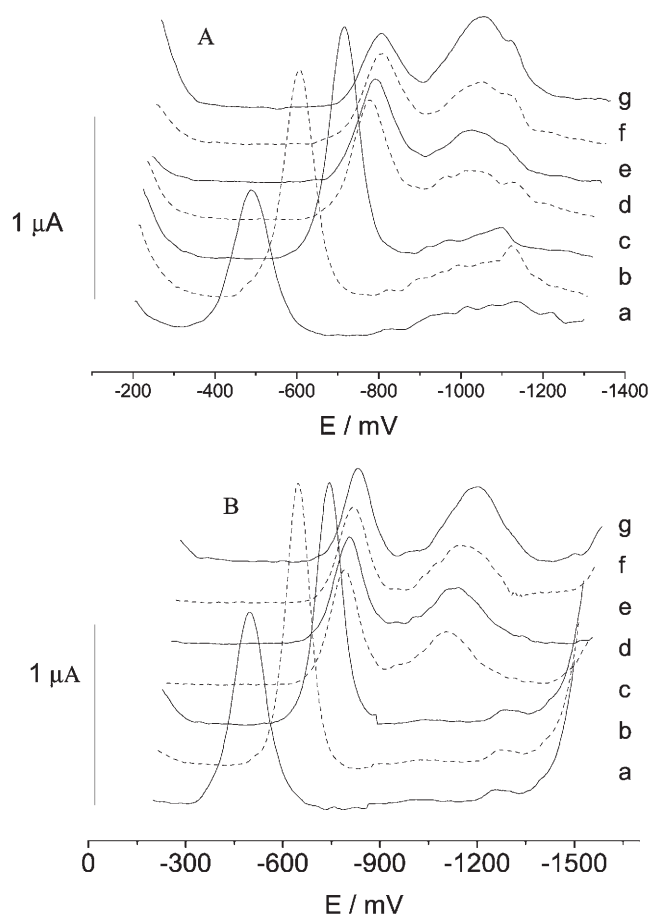

Fig. 4. DPP curves obtained 0.1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer at different pHs between 2 and 11 with: A) 15 mM CTAB; B) 15 mM Hyamine. pH: 1.8 (a), 5.0 (b), 7.5 (c), 8.6 (d), 9.2 (e), 9.7 (f), 10.5 (g), 11.0 (h).
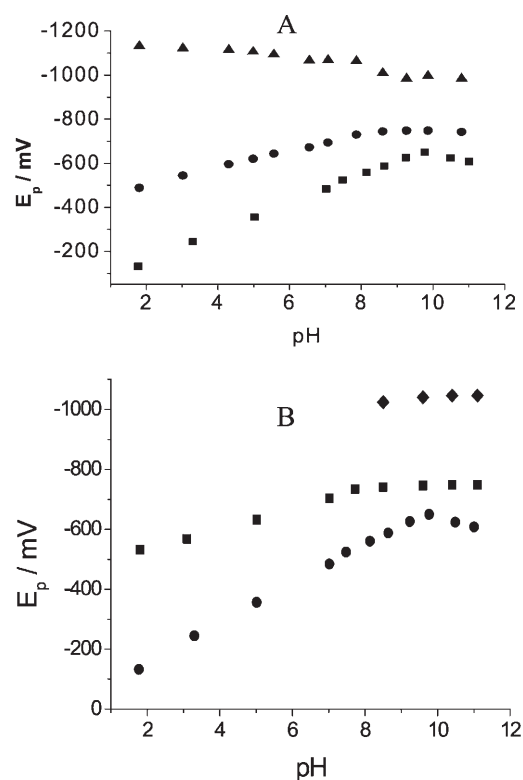
Fig. 5. Comparative E_p vs. pH plots obtained from 0.1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer at different pHs between 2–11 with different surfactants: A) ■ = without surfactants, ● = peak I and ▲ = peak II with 15 mM CTAB; B) ● = without surfactants, ■ = peak I and ◆ = peak II with 15 mM Hyamine.

presence of the surfactants did not change the characteristics of the electrochemical processes, consequently only one DPP peak due to the 4-electron reduction of the nitrogroup was observed with and without surfactants added.

The observed splitting when cationic surfactants were added it was also confirmed by cyclic voltammetry (CV). Figure 6 displays CVs at the same pH by using different surfactants but only in the case wherein cationic surfactants (CTAB and Hyamine) were used it was possible to observe the splitting of the nitroreduction peak according to the above Equations 2 and 3. Notice that in the presence of SDS and Triton, the morphology of the nitroreduction peak is essentially the same as that in its absence, indicating that SDS or Triton-X micelles do not modify the characteristics of the electrochemical processes. According to these results we found the possibility of stabilizing the nitroradical anion at the interfaces of cationic micelles meaning a very good alternative to study this free radical in an aqueous medium. Our results are in accord with the previous results of Saveant et al. [12], which have reported a remarkable stabilization of the anion radical of phthalonitrile in the presence of cationic micelles and suggested that the observed 250-fold decrease in the rate of protonation of the anion radical was due to its association with the positively charged micelles. However,

our results are contradictory with the previously reported by Mc Intire et al. [13] which examined the reductive electrochemistry of nitrobenzene in anionic, cationic and nonionic micelles, and found that the only medium capable of stabilizing the nitroradical anion to the point where it became detectable by cyclic voltammetry was not the cationic, but rather the anionic micelles. The combined results suggest that the influence of micellar systems on the electrochemistry of a particular substrate cannot be predicted only on the basis of simple electrostatic considerations and that micellar effects are strongly dependent upon the substrate and surfactants structures [16]. However, considering that our results show that the nitroradical anion is more persistent in the presence of cationic micelles (Hyamine and CTAB) that in either isotropic aqueous solution or nonionic (Triton-X) or anionic (SDS) micelles an explanation based in electrostatic considerations appear to be more important. Consequently the effect of cationic micelles on the stabilization of the nitroradical anions is: (a) the nitroradical anions are more strongly bound to the cationic micelle than the parent compound due to favorable coulombic interaction; (b) the cationic micelles do protect the anion radical against protonation by water.

Using the preceding cyclic voltammetric response, we can study the kinetic stability of the nitroradical species from 4-MNImOH in a micellar medium. As can be observed from Figure 6 (whole line), in the case of Hyamine and CTAB micelles a very well resolved CV for the nitro/nitroradical anion couple ($RNO_2/RNO_2^{•-}$) can be obtained by adjusting the switching potential appropriately. In Figure 7, we can observe the isolated CV for the $RNO_2/RNO_2^{•-}$ couple from 4-MNImOH solutions containing Hyamine as surfactant at different sweep rates. Furthermore, in the insert of Figure 7, the effect of different sweep rates on the current ratio for solutions having Hyamine as surfactant at three different pHs is shown. A similar general trend in all cationic surfactants was observed, 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 Nicholson–Shain criteria [17]. Furthermore the current ratio was dependent on the 4-MNImOH concentration implying a second-order chemical step. According to the previously observed for the same compound in a mixed medium [14], the second-order chemical reaction is the disproportionation of the nitroradical anion, consequently, we have used the theoretical approach of Olmstead et al. [18] for disproportionation, to calculate the kinetic second-order rate constant, k_2, and the corresponding half-lifetime for the first half-life. The obtained kinetic second-order rate constant, k_2, and the corresponding half-lifetime values for the nitroradical anion from 4-MNImOH in aqueous medium with micellas at different pHs are shown in Table 1. Furthermore we have included the comparison of solutions containing different micellar concentration and solutions in mixed media without micelles. From these results we can state that the use of micellas is the only condition capable to stabilizing the nitroradical anion from 4-MNImOH to the
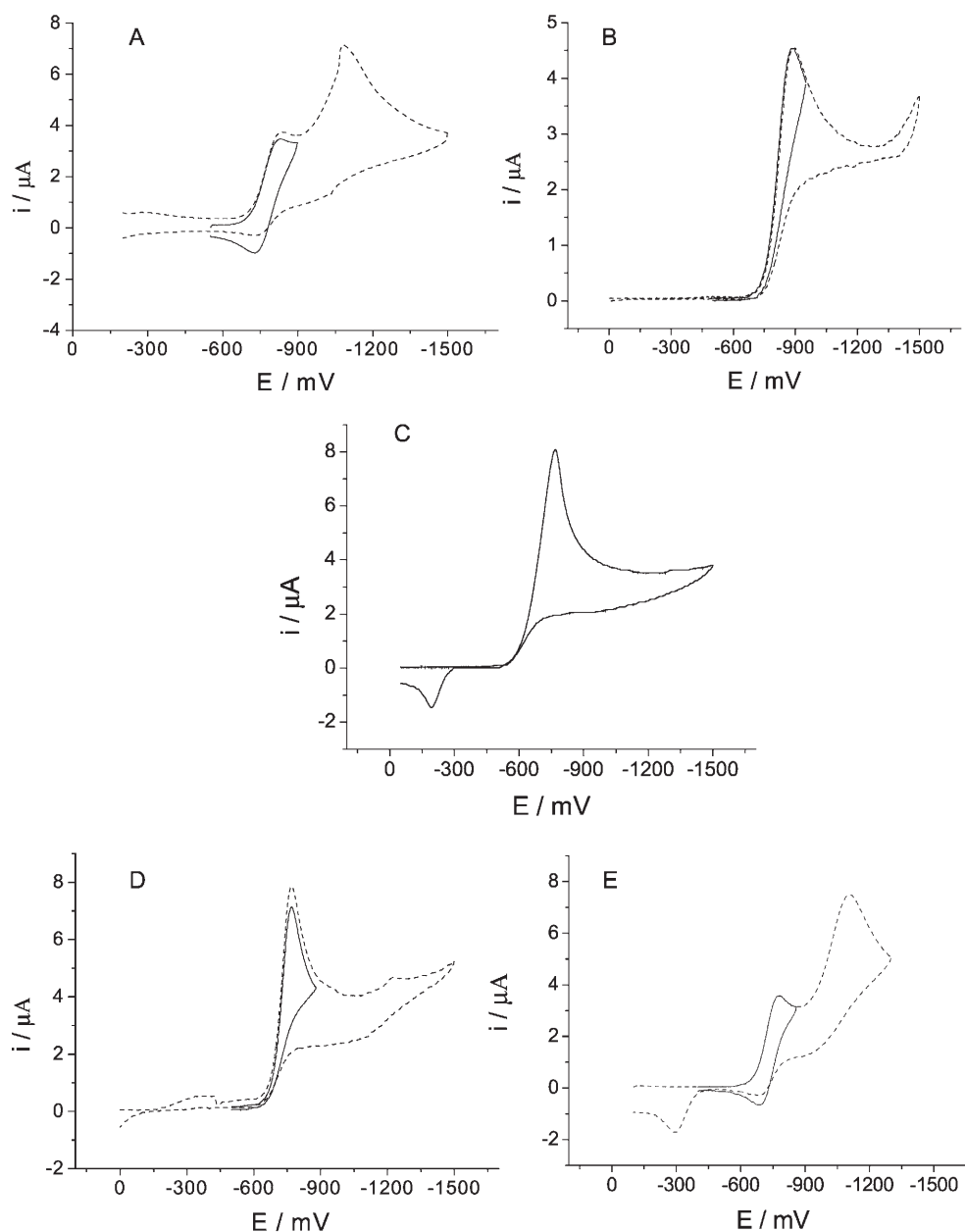
Fig. 6. CVs of 1 mM 4-MNImOH in 0.1 M Britton – Robinson buffer at pH 8.4 containing 15 mM of different surfactants: A) CTAB, B) Triton-X, C) without surfactant, D) SDS and E) Hyamine. Sweep rate = 1 V/s. Whole lines in A), E) shows a short sweep with RNO_2/ $RNO_2{}^{\cdot -}$ couple isolated.

point where it became detectable by CV, in aqueous solution in the pHs 7 – 9 zone. In mixed media without surfactants the CV study it was possible only since pH 10. Probably this fact is due to the decrease in the rate of protonation of the anion radical. On the other hand, when the concentration of Hyamine diminished from 15 to 5 mM the k_2 values shows a six-fold increase and the cathodic peak potential diminishing about 20 mV. Both facts can be rationalized by the adsorption of the surfactants at the electrode surface which may alter the overvoltage of the electrode and by the formation of micellar aggregates which influences the disproportionation reaction. Furthermore, in all the cases,

when micelles were incorporated in the aqueous solution the cathodic peak potentials were cathodically shifting making the reduction of the nitroderivative more difficult. Also, if we compare the results obtained with the same concentrations of micelles but at different pHs we found that the pH affected strongly the stability of the nitroradical anion (k_2 values) however did not affect the energetic of the reduction of the nitrogroup ($E_{p,c}$ values). These results confirmed the pH-dependence of the disproportionation reaction and the pH-independence of the one-electron reduction of the nitroderivative to form the corresponding nitroradical anion according to the above Equation 2.
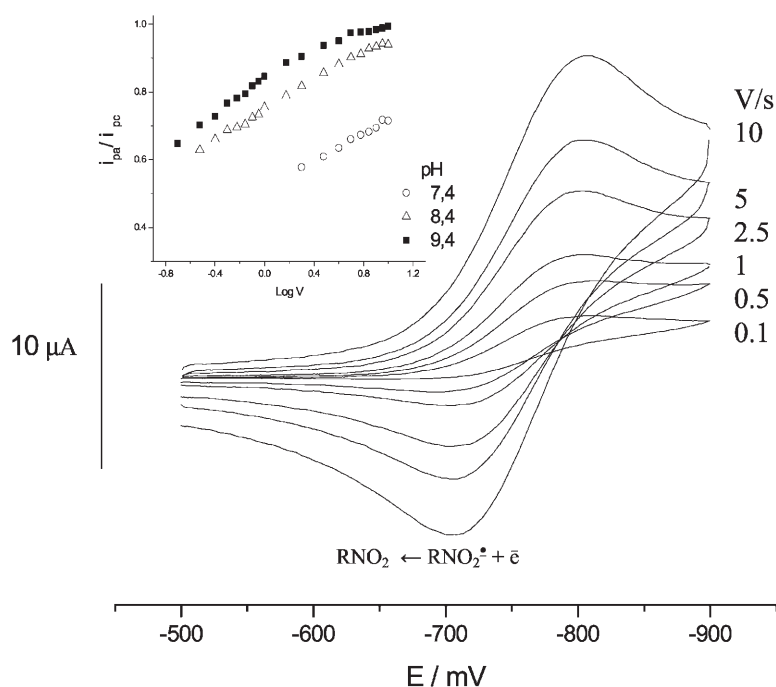
Fig. 7. Cyclic voltammograms showing the $RNO_2/RNO_2^{\cdot-}$ couple of 1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer at pH 8.4 containing 15 mM Hyamine at different sweep rates. Insert: Current ratio dependence on sweep rates of $RNO_2/RNO_2^{\cdot-}$ couple of 1 mM 4-MNImOH in 0.1 M Britton–Robinson buffer containing 15 mM Hyamine at pHs 7.4, 8,4 and 9.4

Table 1. Disproportionation rate constants, half-lifetime for the nitroradical anion, and cathodic peak potentials for the $RNO_2/RNO_2^{\cdot-}$ couple from 4-MNImOH aqueous solutions in 15 mM Hyamine micelles at different pH.

pH	$k_2 \times 10^{-4}$ (L mol^{-1}s^{-1})	$t_{1/2}$ (ms)	$-E_{p,c}$ (mV)
7.4	104 ± 2.11	0.96	804
8.4	4.30 ± 0.43	23.25	802
9.4	1.37 ± 0.21	72.99	796
9.4[a]	8.77 ± 0.62	11.40	774
10[b]	0.59 ± 0.03	169.49	760
10[c]	1.36 ± 0.21	73.52	727

[a] Aqueous solution in 5 mM Hyamine micelles. Data obtained [15] from solutions containing a mixed medium. [b] 30/70: Ethanol/ Britton–Robinson buffer without micelles. [c] 60/40: DMF/citrate buffer, KCl without micelles.

4. Conclusions

We have found that reduction of 4-nitroimidazole derivative in aqueous medium in presence of cationic micelles generated a nitroradical anion sufficiently stable to be detected in the time scale of the cyclic voltammetric technique. This finding provides a very useful tool to quantify the formation, stability and reactivity of the nitroradical anion in conditions that resembling the cell environmental in biological membranes.

5. Acknowledgements

6. References

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