Nitro radical anions from megazol and related nitroimidazoles in aprotic media. A father–son type reaction triggered by an acidic proton

M. Bontá [a], G. Chauviere [b], J. Périé [b], L.J. Núñez-Vergara [a], J.A. Squella [a,*]

[a] *Organic and Physical Chemistry Department, Bioelectrochemistry Laboratory, Chemical and Pharmaceutical Sciences Faculty, University of Chile, Olivos 1007, P.O. Box 233, Santiago 1, Chile*

[b] *Groups de Chimie Organique Biologique, ESA 5068 du CNRS, Université Paul Sabatier, Toulouse, France*

Abstract

We have studied the electrochemical reduction of some nitroimidazoles such as megazol(2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazol, CAS 19622-55-0) and two related derivatives in aprotic media (100% DMF, 0.1 M TBAP). All the studied compounds were easily reducible in aprotic media generating the corresponding nitro radical anions as products of the one electron reduction of the parent compound. The nitro radical anions decay by a dimerization reaction and the dimerization rate constants were obtained according to the Olmstead's approach by obtaining values of 150 ± 24, 1690 ± 42 and 640 ± 32 M^{-1} s^{-1} for megazol, GC-361 and GC-284, respectively. The existence of an acidic proton on the acetamide group in the GC-361 molecule triggered the appearance of father–son type reactions between the nitro radical anion from GC-361 (son compound) and GC-361 (father compound) generating the neutral radical and the conjugate base of GC-361. Thus the nitro radical anion from GC-361 acts as a Brönsted base abstracting the proton of the acetamide group in the GC-361 derivative of megazol.

Keywords: Nitroimidazole derivatives; Nitro radical; Aprotic media

1. Introduction

As a part of a comprehensive study of the search of new therapeutic alternatives for the treatment of Chagas disease (a parasitic illness caused by the pathogen *Trypanosoma cruzi*), we have focused our attention on a series of nitroimidazole compounds related to megazol(2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazol, CAS 19622-55-0). Several authors demonstrated the high efficiency of megazol against *T. cruzi* [1,2] stimulating to test similar compounds as other therapeutical alternatives. Consequently, megazol has become a core structure for the design of new compounds and for the identification of biological targets involved in Chagas disease [3]. The mechanism of action is unknown, but certainly the reduction of megazol is the key in that mechanism. The single electron reduction of megazol by NADPH:Cytochrome P450 reductase, by rat liver as well as by trypanosome microsomes was confirmed by ESR experiments [4,5]. On the other hand, it is well known that biological activity of several nitroimidazoles is dependent upon the nitro group reduction process due to the formation of active intermediate species [6–10] that interact with DNA to produce biochemical damage.

The electrochemistry of nitroimidazoles has been extensively touched in the last years. Generally, those works are focused to the electroanalytical determination of some nitroimidazoles of importance in medicine such as metronidazole [11], ornidazole [12], secnidazole [13] and tinidazole [14]. Furthermore, the use of cyclic voltammetry in order to obtain the lifetimes of nitro radical anions from some nitroimidazoles such as misonidazole and metronidazole [15–18] has been also reported. However, the electrochemical behavior of

E-mail address: asquella@ciq.uchile.cl (J.A. Squella).

megazol has been restricted to a couple of papers from our laboratory. One paper [19]was devoted to the polarographic study of megazol and some related compounds in protic and mixed media but mainly focused to the electroanalytical point of view. Another paper [20]was focused to the cyclic voltammetric study on nitro radical anion formation from megazol and some related nitroimidazole derivatives in mixed media.

Considering that the most important effects of nitroimidazoles occur at the DNA level wherein the environment is mainly aprotic, in this paper we have focused our attention on the electrochemical behavior of megazol in a totally aprotic media. Furthermore, in order to obtain information on the role-played by both the S atom and the amine substituent in the thiadiazol ring of megazol, we have compared two related compounds wherein these components were replaced by O atom and acetamide, respectively.

2. Experimental

2.1. Reagents and solutions

Megazol, GC-361 and GC-284 (Fig. 1) were synthesized and characterized by one of the authors according to a published procedure [21]. All the other reagents employed were of analytical grade.

Stock solutions of each compound were prepared at a constant concentration of 0.025 M in DMF. The polarographic and cyclic voltammetric working solutions were prepared by diluting the stock solution until to obtain final concentrations of 0.5 and 1 mM, respectively.

Dimethylformamide (DMF) and tetrabutyl ammonium perchlorate (TBAP) as solvent and supporting electrolyte were used as aprotic media.

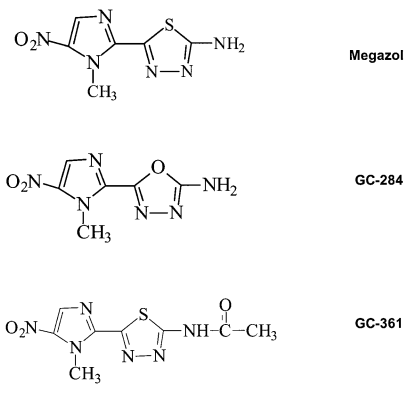


Fig. 1. Chemical structures of megazol and nitroimidazole derivatives.

2.2. Apparatus

Electrochemical experiments were performed with a totally automated BAS CV-50W voltammetric analyzer. All experiments were carried out at a constant temperature of 25 ± 0.1 °C using a 10-ml thermostated cell. A mercury drop electrode (controlling growth mercury electrode, CGME stand of BAS) with a drop area of 0.42 mm^2 as working electrode and a Pt wire as a counter electrode were used. All potentials were measured against Ag | AgCl (3 M).

For differential pulse (DP) and tast polarography, the CGME stand was used in a CGME mode and for cyclic voltammetric experiments, the CGME stand was used as SMDE mode (static mercury drop electrode).

CV curves were recorded with a BAS CV-50W electrochemical system with a CGME, linked to a Gateway 2000 PC for acquisition and data processing. A three electrode cell containing an HMDE as the working electrode, an Ag | AgCl | KCl_{sat} electrode supplied with a bridge for aprotic solvents as the reference electrode. Simulated CV curves were obtained using the software DIGISIM® 2.1 CV simulator for Windows. Software was run using a Gateway 2000 PC.

For the kinetic analysis carried out in aprotic media, the return-to-forward peak current ratio $I_{p,a}/I_{p,c}$ for the reversible one electron couple ($ArNO_2/ArNO_2^{\bullet -}$) was measured for each individual cyclic voltammogram according to the procedure described by Nicholson [22]. The scan rate was varied between 0.1 and 10 V s^{-1}.

Using the theoretical approach of Olmstead et al. for dimerization or dismutation [23,24], the $I_{p,a}/I_{p,c}$ values measured experimentally at each scan rate were inserted into a working curve to determine the parameter ω, which incorporates the effects of rate constant, drug concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by the equation:

$$\omega = k_2 \times C_o \times \tau$$

where k_2 is the second-order rate constant for the decomposition of $ArNO_2^{\bullet -}$. 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. 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.

2.2.1. Controlled potential electrolysis

CPE was carried out either on a Pt coil electrode, pool mercury or a glassy carbon electrode at 200 mV more negative than the first cathodic peak potential of each compound, in DMF containing 0.1 M TBAP as supporting electrolyte. Oxygen was removed by pure,

dry pre-saturated nitrogen. A three-electrode circuit with an Ag | AgCl | KCl_{sat} electrode as reference and Pt wire as the counter electrode was used. A Wenking potentiostat model POS88 was used to electrolyze nitroimidazole derivatives.

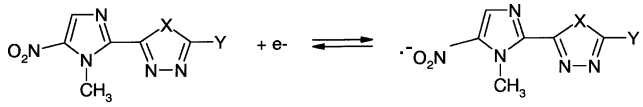
2.2.2. UV–Vis spectroscopic studies

In order to obtain further information on the mechanism, mainly on the progress of the electrolysis, a UNICAM UV-3 spectrophotometer was used. UV–Vis spectra were recorded in the 200–800 nm range at different intervals. Acquisition and data treatment were carried out with the vision 2.11 software. An electrolytic cell of our own construction [25] based on a 1 cm UV cuvette, with a Pt coil as the working electrode was used for the in situ generation of the reduction species. The electrolysis was conducted under constant stirring, which was stopped before each measurement.

3. Results and discussion

Two megazol related compounds have been synthesized in order to study how some selected changes in the structure affect the electrochemical behavior of the nitro radical anion produced in aprotic media. One of these compounds, (GC-361), was selected in order to obtain information about the role played by a group with an acidic proton instead of the amino group. The other one (GC-284) was selected in order to study the effect of replacing the thiadiazol by the oxadiazole moiety.

According to the previously obtained results in protic and mixed media [19,20], all the studied compounds were also easily reduced in aprotic media. In Fig. 2, we can observe the cyclic voltammograms obtained for megazol, GC-284 and GC-361 obtained in 100% DMF with 0.1 M TBAP in both extended and shortened sweeps. All the compounds follow a similar general pattern showing voltammograms wherein it is possible to distinguish at least three main peaks. A first reversible reduction peak, I_c, with a cathodic peak potential at −990 and −1130 mV, depending on the type of nitroimidazole compound, was observed. The respective anodic peak, I_a, appears at −970 and −1042 mV generating a well-defined redox couple due to the one electron reduction of the nitro group according to the following well-known equation.



Megazol	X = S	Y = NH_2
GC-284	X = O	Y = NH_2
GC-361	X = S	Y = $NHCOCH_3$

(1)

The corresponding cathodic peak potentials and the differences between anodic and cathodic peak potentials are shown in Table 1. As can be observed in Tables 1 and 2, the replacement of the S atom by an O in the diazole ring of megazol molecule did not markedly affect neither the easiness of reduction of the nitro group nor the stability of the nitro radical anion. According to the previously found, in mixed and protic media [19,20], the replacement of S atom by O in this molecule affect only the adsorption properties which are non-existent in this aprotic media. Furthermore, we have carried out coulometric experiments finding that the number of electrons transferred was slightly greater than one, as shown in Table 1. The obtained ΔE_p values are significantly greater than the 60 mV expected for a one

Table 1
Cathodic peak potentials and anodic–cathodic peak potential differences obtained from cyclic voltammograms of 1 mM solutions of nitroimidazole compounds in DMF containing 0.1 M TBAP on HMDE at 1 V min^{-1}

Compound	$-E_{p,c}$ (mV)	ΔE_p (mV)	n
Megazol	990	87.6	1.30±0.15
GC-361	1130	71.2	1.17±0.17
GC-284	1025	62.5	1.20±0.15

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

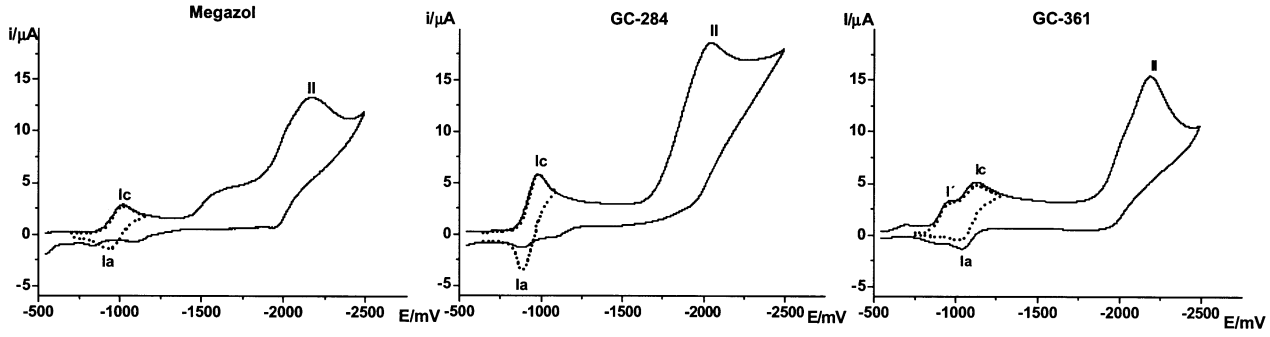
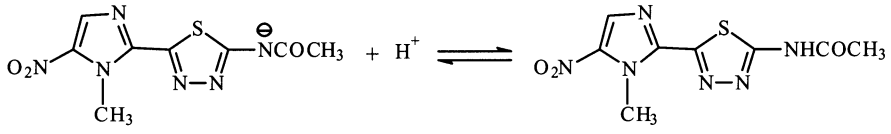


Fig. 2. Cyclic voltammograms of 1 mM megazol, GC-284 and GC-361 in 100% DMF with 0.1 M TBAP. Sweep rate 1 V s^{-1}. Dotted line shows a short sweep with the isolated first couple.

Table 2
Second-order rate constants for the nitro radical anion chemical decay step obtained from the Olmstead's approach assuming both dimerization and dismutation

Compound	$k_{2,Dim}$ (M^{-1} s^{-1})	$k_{2,Dism}$ (M^{-1} s^{-1})
Megazol	150 ± 24	1126 ± 42
GC-361	1690 ± 42	4996 ± 64
GC-284	640 ± 32	1736 ± 40

electron reversible transfer. Probably, this deviation can be ascribed as a loss of reversibility, going to a Quasi-reversible process in the case of megazol and GC-284. At a more cathodic potential, i.e. over −2000 mV, a second cathodic wave II appears. This cathodic wave is irreversible, corresponding to the reduction of the nitro radical anion to the nitroso dianion derivative as has been previously informed for other nitroaromatic compounds in aprotic media [26,27]. In the case of the cyclic voltammogram of megazol, we can observe a shoulder between the peaks I_c and II probably due to the three electron reduction of the protonated nitro radical, as has been proposed by Baumane et al. [26]. In this paper, we have focused our attention on the behavior of the reversible couple due to the one electron reduction of the nitro group to form the nitro radical anion. This couple is very well defined when a shortened scan was run (Fig. 2, dotted line). Single observations of the cyclic voltammogram of the reversible couple permits to appreciate a big difference between the behavior of both megazol and GC-284 and the other compound GC-361. In fact, in the case of GC-361 the voltammogram reveals a notorious distortion showing a pre-peak, *I*. However, it was observed that this pre-peak completely disappeared when a strong base such as a NaOH saturated ethanol solution was added (Fig. 3A). Identical behavior can be followed more quantitatively by DP polarography. In Fig. 3B, we can observe the DP polarogram corresponding to the nitro group reduction of GC-361 obtained in 100% DMF with 0.1 M TBAP. On the other hand, if we add an acid such as acetic acid in ethanol solution to the above solution wherein the pre-peak was eliminated, the phenomena were reverted generating the pre-peak again (Fig. 3C). Obviously, these experiments lead us to consider the existence of an acid–base equilibrium in the aprotic media. Furthermore, considering that the above phenomenon does not occur in the case of megazol (wherein the only difference is the amino instead of an acetamido group in the thiadiazol moiety), we suppose that the proton involved in the acid–base equilibrium must be the proton on the acetamido group of GC-361. Consequently, we propose that the equilibrium occurring in aprotic media in the case of GC-361 can be described as follows:



(2)

Considering this equilibrium as a slow occurrence, we can explain the pre-peak as the reduction of the uncharged species, with the negative charged species

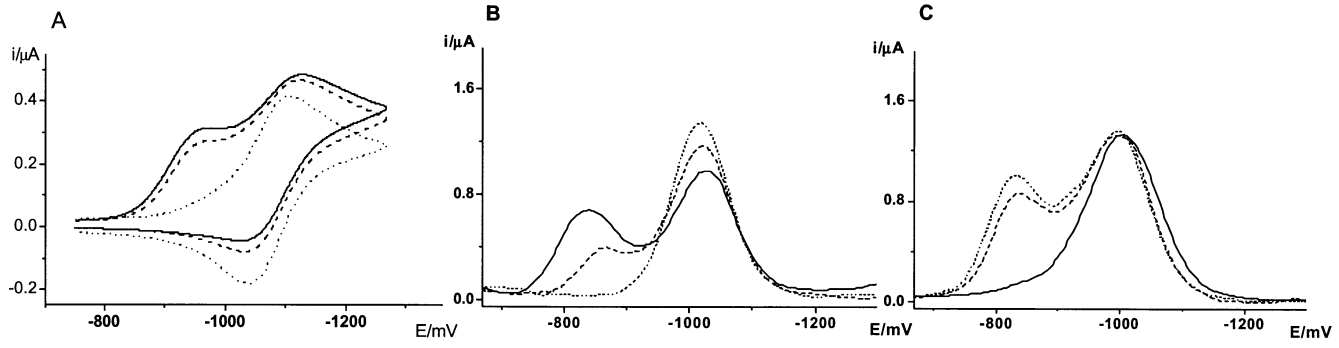


Fig. 3. (A) Cyclic voltammograms 0.5 mM GC-361 plus different quantities of NaOH saturated ethanol solution: —, without; – – –, 0.2 mM; and ···, 0.8 mM. (B) DP polarograms of 0.5 mM GC-361 plus different quantities of NaOH saturated ethanol solution: —, without; – – –, 0.16 mM; and ···, 0.32 mM. (C) DP polarograms of 0.5 mM GC-361 with NaOH saturated ethanol solution plus different quantities of acetic acid saturated ethanol solution: —, without; – – –, 0.32 mM; and ···, 0.4 mM.

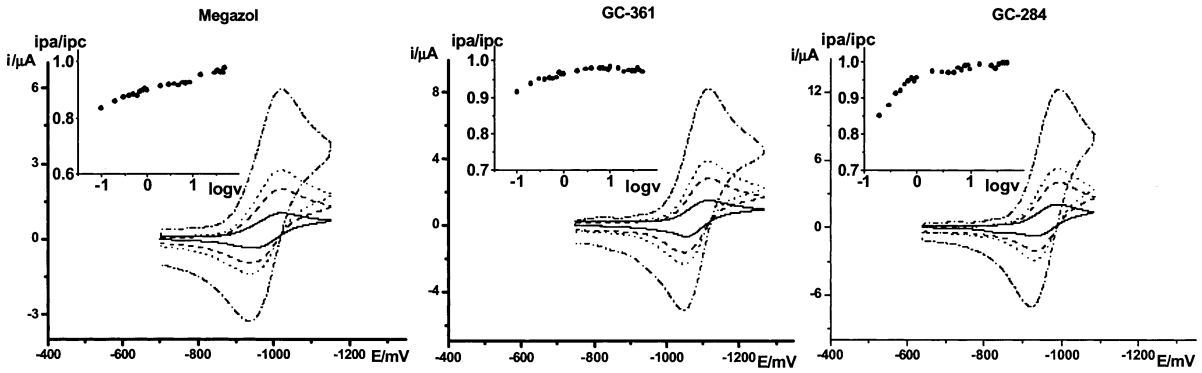


Fig. 4. Cyclic voltammograms showing the reversible couple to the one electron reduction of nitroimidazoles at different sweep rates between 0.1 and 50 V s^{-1}: —, 0.1; – – –, 0.5; ···, 1; ·–·, 5 V s^{-1}. Inset: current ratio dependence on sweep rate for the same experiments.

being reduced at higher cathodic potentials. In order to obtain non-distorted cyclic voltammograms for GC-361, we have been working by adding a few microliters of NaOH saturated ethanol solution in the media.

Using the cyclic voltammetric response of the nitro/nitro radical anion redox pair, we can study the kinetic stability of the nitro radical anion species of all studied compounds. In Fig. 4, we can observe the effect of

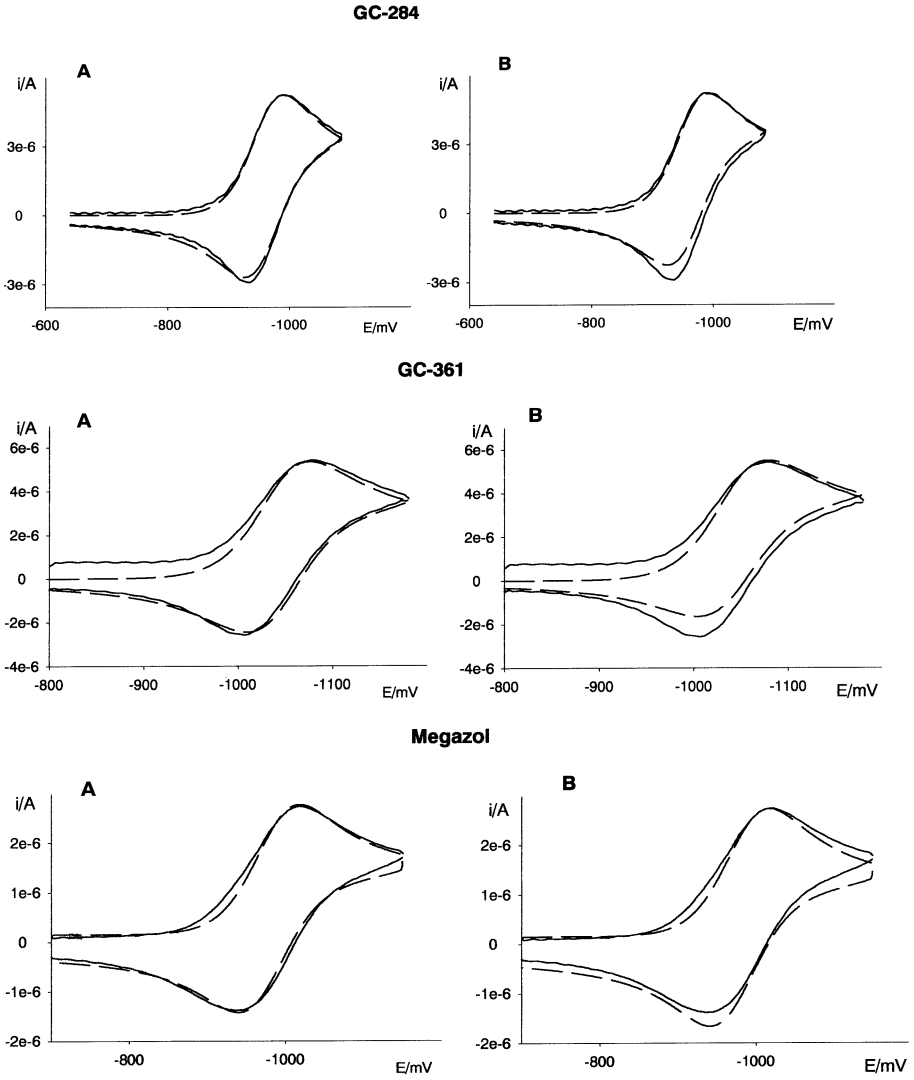


Fig. 5. Comparison of experimental (whole line) and simulated (dashed line) cyclic voltammograms for all the studied nitroimidazole derivatives considering either: (A) dimerization; or (B) dismutation as the chemical coupled reaction.

different sweep rates on the corresponding cyclic voltammograms showing that all the compounds follow a similar general tendency. As can be seen, the $I_{p,a}/I_{p,c}$ current ratio increases as the scan rate is increased, reaching a limiting value of 1. These results fulfil the requirements for an irreversible chemical reaction following a reversible charge transfer step [28], but we have also found that the current ratio depends on the nitroimidazole derivative concentration, implying a second-order chemical step. According to our previous studies in mixed media [20], the second-order chemical step corresponds to a dismutation reaction but in this aprotic media, we have tried both dismutation and dimerization. Using the theoretical approach of Olmstead et al. for dismutation [23] and dimerization [24], we have calculated the second-order rate constant, k_2, obtaining different values depending if dismutation or dimerization was assumed (Table 2). To decide which type of chemical reaction depict better than the real mechanism, a simulation procedure was used. The simulation procedure involved simulating cyclic voltammograms using alternatively either the obtained dismutation or dimerization rate constant and then compare the simulated cyclic voltammograms with the experimental ones. In Fig. 5, we can observe a comparison of experimental and simulated cyclic voltammograms for all the studied nitroimidazole derivatives considering either dismutation or dimerization as the chemical reaction. From these results, it can be appreciated that in the case wherein dimerization was considered as the

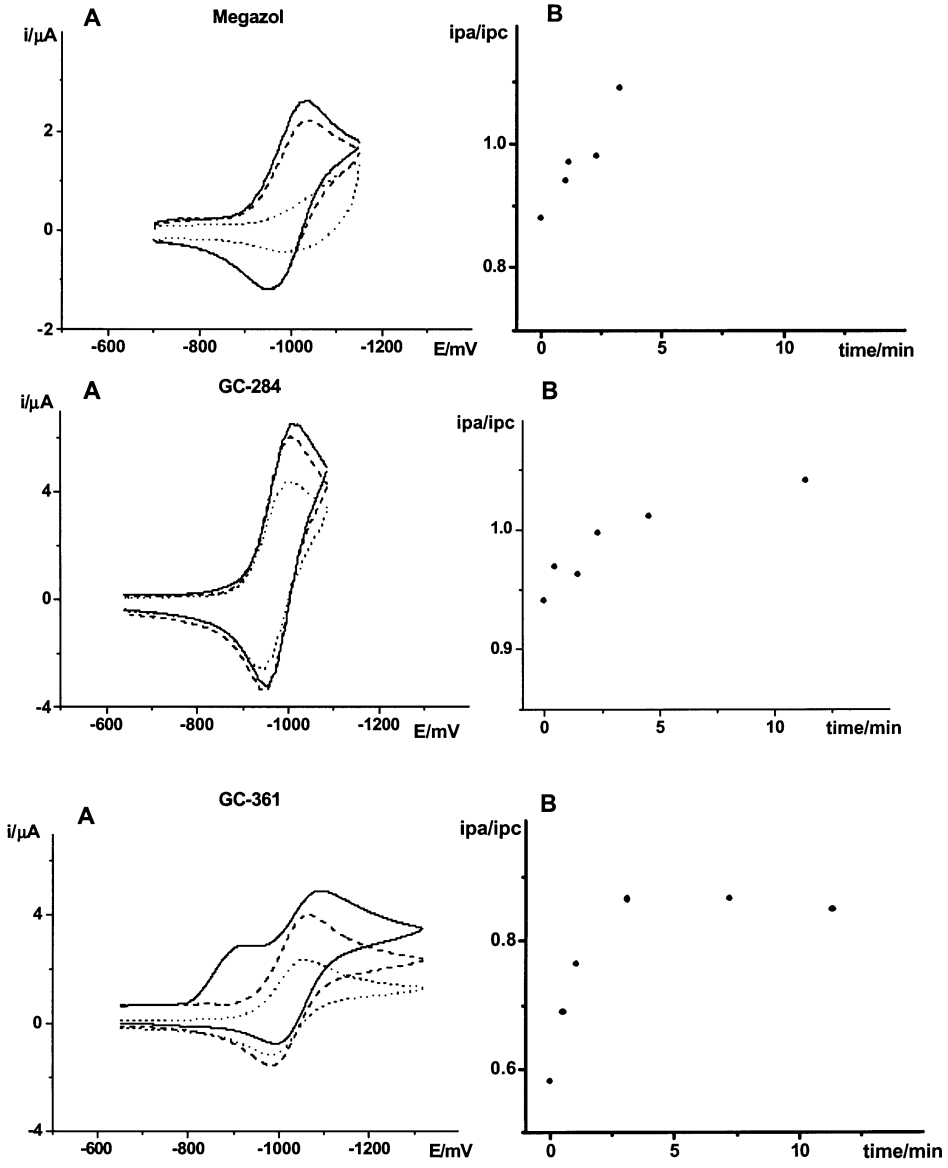


Fig. 6. (A) Evolution of the nitro/nitro radical anion couple; and (B) the evolution of the peak current ratio at different CPE times: —, 0; – – –, 1.1; and ···, 11.3 min.

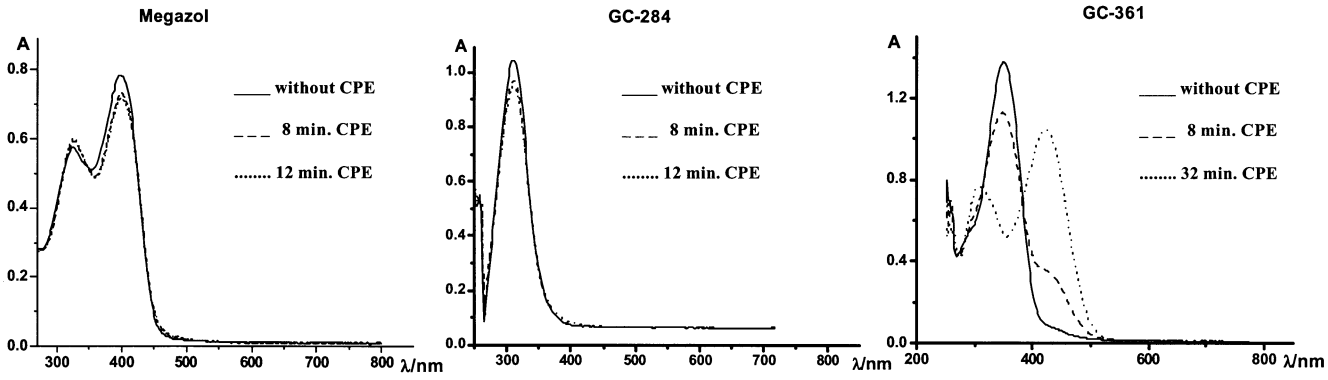


Fig. 7. Time-course of CPE followed by UV–Vis spectrum for all nitroimidazole compounds.

chemical step, the fit between simulation and experimental curves was clearly better. Specifically, simulated curves in the case of dismutation reaction originate markedly different currents in the anodic peaks than the experimental curves, meaning that the chemical decay of the nitro radical anion was wrongly estimated. On the other hand when a dimerization reaction was considered, the fit between experimental and simulated voltammograms was perfect meaning that, in aprotic media, the nitro radical anion decays according to a dimerization reaction. This finding differs with the earlier described in mixed media [20] meaning that a change in the proton activity and the polarity character of the medium produces not only a change in the stability of the radical anion but rather a change in the mechanism changing from a dismutation in mixed media to a dimerization in aprotic media. In spite of the main evidences from our results, it leads us to think in a dimerization reaction that we cannot totally discard a concomitant occurrence to some extent of both pathways, dimerization or dismutation.

In order to obtain further information about the behavior of the nitro/nitro radical anion couple, we have submitted all the nitroimidazole solutions to controlled potential electrolysis experiments followed by cyclic voltammetry and UV–Vis spectrophotometry. We have selected a controlled potential value approximately 200 mV more cathodic than the peak potential of the I_c peak, in such way of generating exhaustively the corresponding nitro radical anion. As is expected, we observed a diminishing of both $I_{p,a}$ and $I_{p,c}$ when the electrolysis time was increased, as a consequence of the reduction of the nitro group. Simultaneously, we can observe an increase in the peak current ratio $I_{p,a}/I_{p,c}$ meaning the existence of higher quantity of nitro radical anion capable to be oxidized in the anodic sweep due to the electrolysis generation. In Fig. 6, we can observe the evolution of the nitro/nitro radical anion couple with the time-course of the electrolysis and the evolution of the peak current ratio between the first 15-min of the electrolysis. From the above results, it appears that the evolution of the couple was rather different in the case

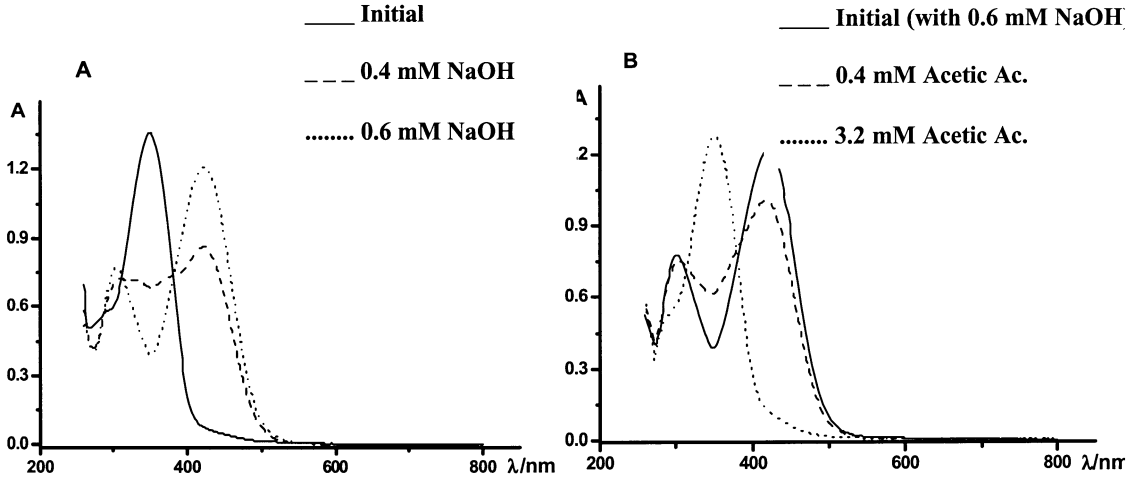
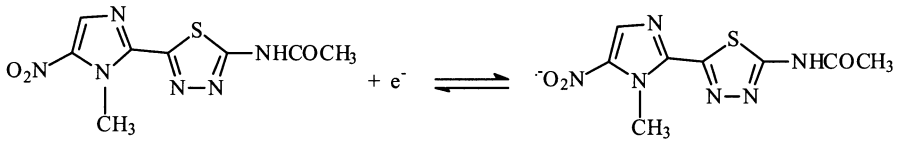
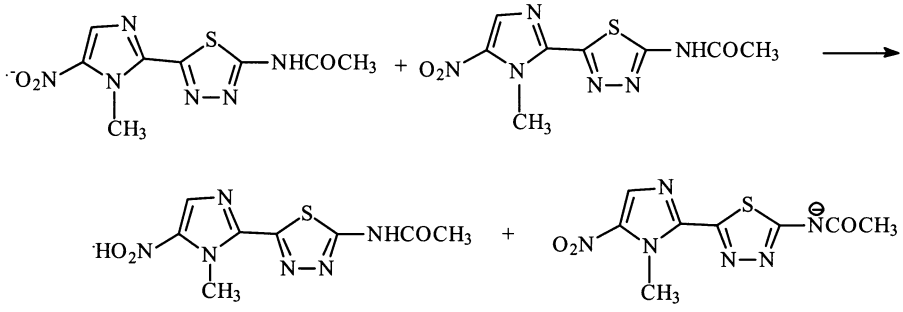


Fig. 8. UV–Vis spectra of GC-361 solution after adding: (A) different quantities of NaOH saturated ethanol solution; and (B) the reverted effect by adding different quantities of acetic acid saturated ethanol solution.

of the GC-361 compound. In fact, we can appreciate that the pre-peak disappears during the course of the electrolysis meaning that the uncharged species from the equilibrium of Eq. (2) was consumed. We postulate that this disappearance can be explained taking into account the acid–base properties of the GC-361, in fact, the primary radical anion generated in the electrolysis should be rapidly protonated by the starting GC-361 compound through the father–son reaction with the formation of conjugate base and the neutral radical according to the following equations:



(3)



(4)

Consequently, the occurrence of the electrolysis produces the consumption of the neutral species of GC-361, according to Eq. (3), and the appearance of the charged species of GC-361, according to Eq. (4), explaining the disappearance of the pre-peak in the cyclic voltammogram.

On the other hand, UV–Vis curves of the time-course of electrolysis were also recorded (Fig. 7). We can see that the absorption band at $\lambda_{max} = 330$ and 402 nm for megazol, and the absorption band at 312 nm for GC-284 decrease during the electrolysis, but in the case of GC-361 the behavior was totally different. As can be observed in Fig. 7, the spectra of GC-361 before the electrolysis procedure shows one absorption band at approximately 350 nm but when the electrolysis was carried out, this band decreased and a new absorption band at 424 nm appeared. Surprisingly, this effect in the spectra as a consequence of the electrolysis procedure, can be reproduced by the addition of a strong base such as NaOH saturated ethanol solution to the GC-361 solution (Fig. 8). Consequently, the shifting of the absorption band from 350 to 424 nm can be ascribed to the acid–base equilibrium on the GC-361 molecule as has been previously described in the above equation [2], wherein the absorption bands at 350 and 424 nm corresponding to the neutral and charged species, respectively. The above experiments permit to conclude that the electrolysis procedure produces a change in the equilibrium from the neutral species to the negative charged species giving support to the above proposal in Eqs. (3) and (4).

4. Concluding remarks

All the studied nitroimidazole compounds were electrochemically reduced with generation of the nitro radical anion, but in different way to the previously informed in protic or mixed media because the nitro radical anion decays by dimerization better than dismutation.

The replacement of the S atom in the thiadiazol moiety of megazol did not show influence in both the easiness of reduction of the nitroimidazole compound and the stability of the nitro radical anion in aprotic media. However, the replacement of the amine group in the thiadiazol moiety of megazol affects considerably the reduction of the nitro group giving rise to a father–son type reaction triggered by the existence of an acidic proton in the acetamide group on the thiadiazol moiety. In fact, in this aprotic media, the nitro radical anion species from GC-361 (son compound) acts as a Brönsted base deprotonating GC-361 (father compound).

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