Cyclic voltammetric studies on nitro radical anion formation from megazol and some related nitroimidazole derivatives

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Abstract

Megazol (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazol, CAS 19622-55-0) and related nitroimidazole compounds are being tested as antichagasic drugs. Little is known on the mode of action of megazol. However, there is evidence that one-electron reduction of megazol to the corresponding nitro radical anion is a key step in the reaction mechanism. Consequently, this paper is focused on the cyclic voltammetric behaviour of megazol and related nitroimidazole derivatives with the aim of revealing the formation and stability of the corresponding nitro radical anions. All the compounds studied produce a well resolved nitro/nitro radical anion couple. The resolution of the couple was improved with the addition of tetrabutylammonium ions which hinders the protonation of the nitro radical anion at the electrode surface, thus enhancing the stability of the nitro radical anion. Only megazol produced a cyclic voltammogram distorted by the presence of a pre-peak due to strong adsorption of the corresponding nitro radical anion. The pre-peak occurs at potentials more positive than the diffusion controlled peak because the Gibbs energy of adsorption of the nitro radical anion makes the reduction of megazol to the adsorbed nitro radical anion easier than to the radical anion in solution. The sulphur atom in the thiadiazole ring plays a crucial role in the adsorption phenomena. Using the cyclic voltammetry theory for the disproportionation reaction, we have calculated the second-order decay rate constant, k_2, and the half-life time, $t_{1/2}$, for all the nitro radical anions of the studied nitroimidazole derivatives. The values obtained were compared with those of the corresponding nitro radical anions obtained from nifurtimox and benznidazole, the classic antichagasic drugs. Also, our results show that cyclic voltammetry is a good alternative to the classic pulse radiolysis method to obtain reliable values of the E_7^1 parameter for nitro radical anions.

Keywords: Nitroimidazole derivatives; Nitro radical anion; Cyclic voltammetry

1. Introduction

Megazol (1-methyl-2-(5-amino-1,3,4-thiadiazole)-5-nitroimidazole) was synthesised in the 1960s [1] and was shown to have great antiprotozoan and antibacterial activity [2]. However, it is only recently that megazol and related compounds (Fig. 1), have been tested as antichagasic drugs as a viable alternative to nifurtimox and benznidazole which are being discontinued due to their toxicity. The Chagas disease (American trypanosomiasis) caused by the pathogen *Trypanosoma cruzi*, is one of the many parasitic illness existent today for which it has not been possible to find a satisfactory pharmacological treatment, thus constituting a serious problem of public health at world level [3].

Several authors demonstrated the high efficiency of megazol against *T. cruzi* [4,5]. Others have also demonstrated that this compound is highly active against *Trypanosoma brucei* [6,7] and its pharmacokinetics, metabolism and excretion in animals have been published [8,9]. Currently little is known on the mode of action of megazol. Initial reports that bioreduction had limited association in its action against *T. cruzi* [10] have been challenged, since the drug has been shown to be subject to single-electron reduction by a variety of enzymatic processes with reductase activity [11,12], including *T. cruzi* extracts [12]. Specifically, the single-electron reduction of megazol by NADPH:cytochrome P-450 reductase, by rat liver as well as by *trypanosome*

microsomes has been confirmed by ESR experiments [12]. Consequently, the one-electron reduction of megazol in order to form the corresponding nitro radical anion appears to be a key step in the reaction mechanism.

The biological activity of nitroimidazoles is dependent upon the nitro group reduction process due to the formation of active intermediate species [13,14] that interact with DNA to produce biochemical damage. Several studies dealing with stabilisation of intermediate nitro species during the electroreduction of nitroimidazole derivatives have been done. Barety et al. [15] observed the nitro radical anion by using cyclic voltammetry in non-aqueous medium. The electrochemical production of the nitro radical anion has also been the target of studies of other pharmacologically active compounds, such as nifurtimox [16], calcium antagonist compounds [17–19], nitro-substituted amphetamine derivatives [20], flutamide [21], nitrofurantoin [22] and nimesulide [23]. However, to date, the electrochemical production of the nitro radical anion of megazol has not been reported. In previous work, our laboratory reported the polarographic reduction of megazol [24] but that study focused only on the electroanalytical

point of view without studying the production and stability of the nitro radical anion.

After showing that megazol in the *trypanosome* undergoes a bioreduction leading first to a radical anion which then gives oxygen activated species [12], it was considered worthwhile to synthesise analogous compounds where substituents may change the stability of the nitro radical anion, and therefore improve its redox-cycle properties. Consequently, in this study, we have synthesised megazol and a series of related compounds, shown in Fig. 1, in order to study the formation and stability of the corresponding nitro radical anion.

2. Experimental

2.1. Materials

All the compounds were synthesised and characterised by one of us according to a published procedure [25]. Benznidazole and Nifurtimox were obtained commercially from Laboratorios ROCHE Quimicos e Farmaceuticos S.A., Brazil. All the other reagents
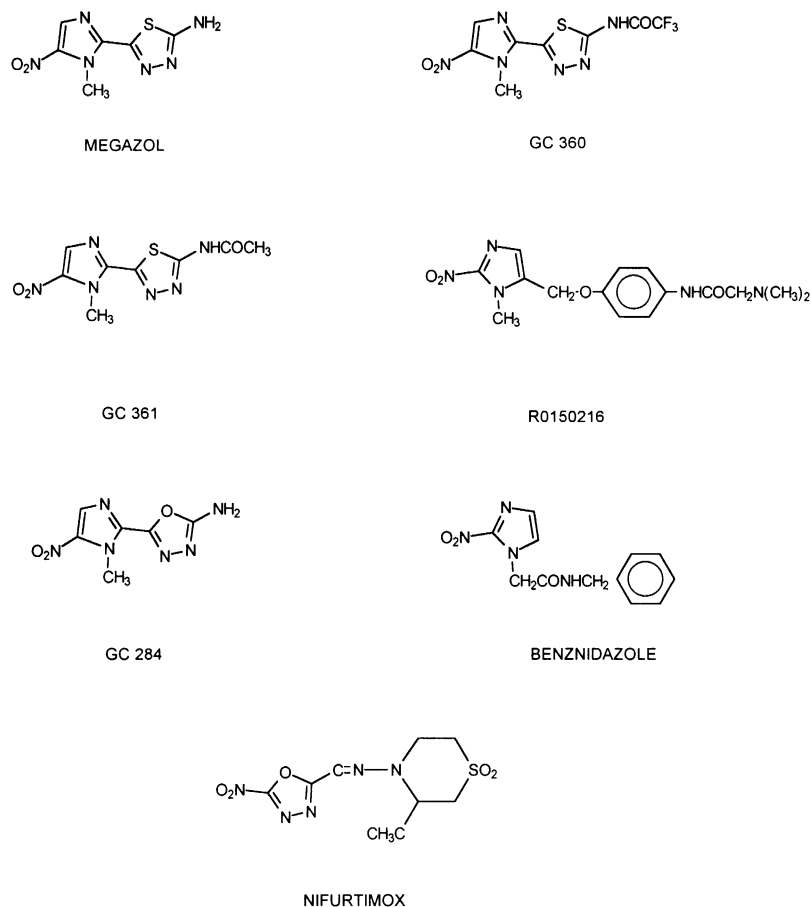

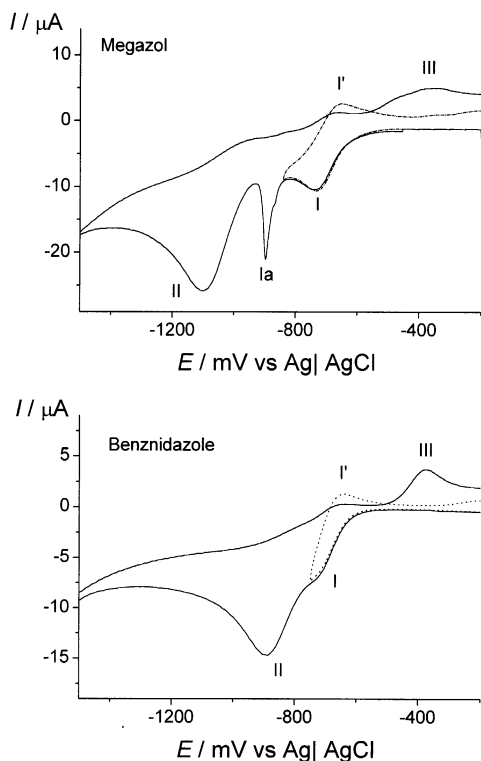Fig. 1. Chemical structures of megazol, several nitroimidazole compounds and nifurtimox.

Fig. 2. Cyclic voltammograms of 5 mM megazol and benznidazole in mixed media: 60/40 DMF + citrate + 0.3 M KCl. Sweep rate 1 V s^{-1}. Dotted line shows a short sweep with the isolated first couple.

employed were of analytical grade without further purification.

1 or 5 mM drug solutions in two different media were used for the voltammetric studies. For the mixed medium a mixture of 60/40 DMF + citrate buffer was used with 0.3 M KCl and 0.1 M TBAI as supporting electrolytes. The pH was adjusted with small aliquots of concentrated NaOH or HCl respectively.

Dimethylformamide (DMF) and tetrabutyl ammonium iodide (TBAI) as solvent and supporting electrolyte, respectively, were used in aprotic media.

2.2. Apparatus

Electrochemical experiments were performed with a totally automated BAS CV-50 W voltammetric analyser. All experiments were carried out at a constant temperature of $25 \pm 0.1°C$ using a 10 ml thermostated cell. A static mercury drop electrode (SMDE mode in a controlling growth mercury electrode stand from BAS) with a drop area of 0.42 mm^2 was used as the working electrode and a platinum wire as the counter electrode. All potentials were measured against Ag | AgCl | 3 M KCl.

2.3. Methods

The pH measurements were corrected according to the following equation [26]: pH* − B = log U_H°, where

pH* equals $-\log a_H$ in the mixed solvent, B is the pH meter reading and the term log U_H° is the correction factor for the glass electrode, which was calculated from the different mixtures of DMF and aqueous solvent, according to a previously reported procedure [27].

In the kinetic analysis carried out in mixed media, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible first-electron transfer (the ArNO$_2$/ArNO$_2^{\bullet-}$ couple) was measured for each cyclic voltammogram, varying the scan rate from 0.1 to 10 V s^{-1} according to the procedure described by Nicholson [28].

Using the theoretical approach of Olmstead and Nicholson [29], 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 rate constant, drug concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by the equation

$$\omega = k_2 c_0 \tau$$

where k_2 is the second-order rate constant for the decomposition of ArNO$_2^{\bullet-}$, c_0 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.
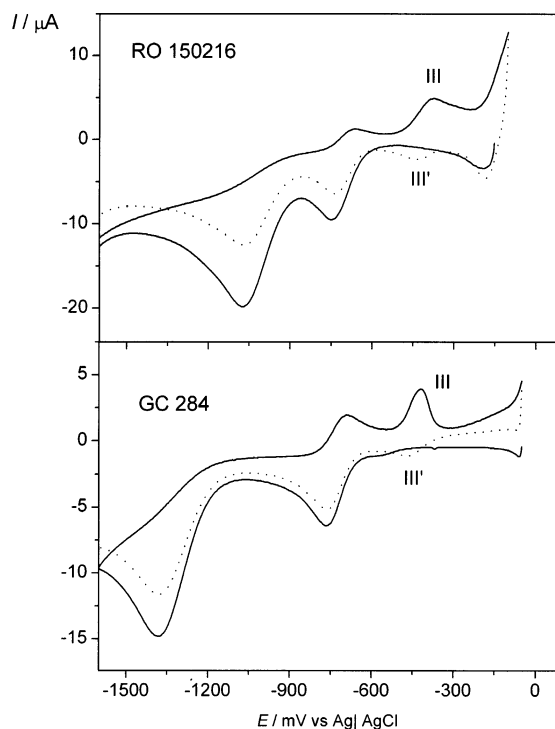

Fig. 3. Cyclic voltammograms of 5 mM RO 150216 and GC-284 in mixed media: 60/40 DMF + citrate + 0.3 M KCl. Sweep rate 1 V s^{-1}. Solid line shows the first sweep and dotted line shows the second sweep.

Table 1
Effect of adding TBAI on the reduction potentials of megazol and related compounds

	Without TBAI		TBAI added	
	E_{pc}^I/mV	$\Delta E_{pI–II}$/mV	E_{pc}^I/mV	$\Delta E_{pI–II}$/mV
Megazol	−728	367	−733	417
GC-361	−765	535	−758	621
GC-360	−745	393	−753	481
GC-284	−734	476	−742	545
Benznidazole	−735	156	−730	411
RO 150216	−729	338	−740	543

3. Results and discussion

3.1. Nitro radical anion formation

As was described in previous work [24], all compounds were electrochemically reducible at a dropping mercury electrode using polarographic modes. The main electrodic response involved the nitro reduction being totally dependent of the medium, varying from (a) one peak due to a four-electron four-proton transfer to form the hydroxylamine derivative, in protic media, to (b) one peak due to one-electron transfer to form the nitro radical anion with a subsequent second irreversible peak due to three-electron four-proton transfer to form the hydroxylamine derivative, in mixed media. However, considering that our current interest is

devoted to the study of the nitro radical anion, we have focused our studies on a medium where this radical is kinetically stable, i.e. mixed or aprotic medium. Furthermore, considering our previous experience [17,18,22,23], we have selected the cyclic voltammetric technique as a very useful tool to study the nitro radical anion in this type of compound.

In Fig. 2, cyclic voltammograms of megazol (5-nitro imidazole-diazole derivative) and benznidazole (2-nitro imidazole derivative) in mixed media containing 60/40 DMF + citrate buffer (pH 9.0, 0.3 M KCl) are shown. For both compounds, behaviour analogous to that in the polarographic mode was observed. Megazol produces two signals, peak I due to the one-electron nitro reduction to form the nitro radical anion, and peak II due to the nitro radical anion reduction to form the hydroxylamine derivative. The peak I′ corresponds to the anodic peak of the nitro/nitro radical anion couple. When the amplitude of the voltammogram is sufficient to permit hydroxylamine derivative formation (solid line in Fig. 2) a second anodic signal, peak III, is observed in the reverse scan. Thus we can conclude that this signal corresponds to the oxidation of the hydroxylamine derivative to the corresponding nitroso derivative. The above described behaviour was observed for all the compounds, but in the case of the 2-nitro imidazole derivatives (benznidazole and RO-150216) the separation between the peak I and peak II potentials ($\Delta E_{pI–II}$) is too small to permit a good resolution of the I/I′ couple (Fig. 2). Probably, this is due to the fact that the protonation of the nitro radical anion on
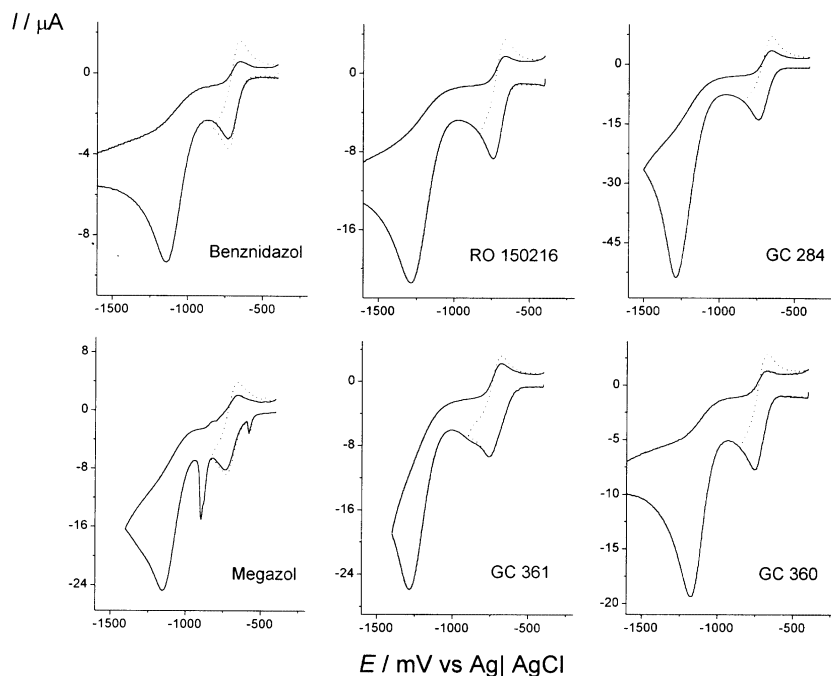

Fig. 4. Cyclic voltammograms of all nitrocompounds (5 mM solution) in mixed media: 60/40 DMF + citrate + 0.3 M KCl + 0.1 M TBAI. Sweep rate 1 V s^{-1}. Dotted line shows a short sweep with the isolated first couple.
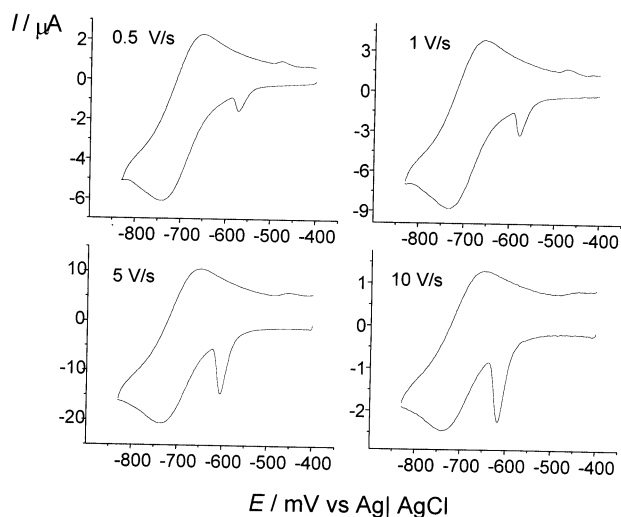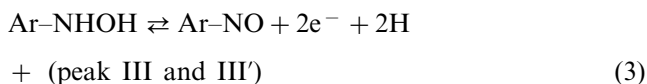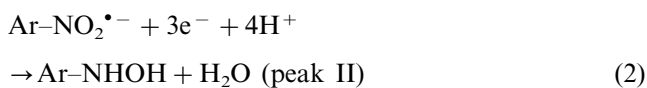
Fig. 5. Cyclic voltammograms of the isolated first couple of megazol (5 mM solution) in mixed media (60/40 DMF + citrate + 0.3 M KCl and 0.1 M TBAI) at different sweep rates.

the electrode surface is more favoured in the 2-nitro imidazole derivatives than in 5-nitro imidazole-diazole derivatives, thus favouring the subsequent three-electron reduction.

On the other hand, the voltammogram of megazol (Fig. 2) shows a very sharp post peak, I_a, probably due to adsorption effects. According to the theory developed by Wopschall and Shain [30], this post peak can be attributed to a reactant strongly adsorbed; in our case megazol is strongly adsorbed on the mercury electrode. Curiously, if we compare the voltammograms of megazol with the related compound named GC-284 (Fig. 3) we can observe that only megazol is strongly adsorbed, leading to the conclusion that the presence of the sulphur atom in the diazole ring is crucial to the adsorption of the megazol molecule.

Furthermore, when a second sweep was run (dotted line in Fig. 3), it was possible to observe that a new couple, (signals III and III′) is generated at lower potentials; this fact confirms that these signals correspond to the quasi-reversible hydroxylamine/nitroso redox couple.

Summarising, the signals that are observed in this medium correspond to the well known mechanism for the nitro aromatic reduction:

$$Ar\text{–}NO_2 + e^- \rightleftarrows Ar\text{–}NO_2^{\bullet -} \quad \text{(peaks I and I′)} \quad (1)$$

$$Ar\text{–}NO_2^{\bullet -} + 3e^- + 4H^+$$
$$\rightarrow Ar\text{–}NHOH + H_2O \quad \text{(peak II)} \quad (2)$$

$$Ar\text{–}NHOH \rightleftarrows Ar\text{–}NO + 2e^- + 2H$$
$$+ \quad \text{(peak III and III′)} \quad (3)$$

3.2. Nitro radical anion stability

In order to improve the resolution of the nitro/nitro radical anion couple and according to previous experience [31], a second supporting electrolyte such as TBAI was added to the solution. The tetraalkylammonium ions are adsorbed at the electrode, where they form a layer with a low proton activity [32]. We used this property in order to hinder the protonation of the nitro radical anion at the electrode surface and thus enhance the kinetic stability of the nitro radical anion, and consequently hinder the subsequent three-electron reduction. In Table 1 we can appreciate the effect of adding TBAI on the cathodic peak potential of peak I and on the separation between peaks I and II. As a consequence of the addition of TBAI a considerable increase in $\Delta E_{\text{pI–II}}$ resulted in a much better resolution of the nitro/nitro radical anion couple. Also, we can appreciate that the enhancement of $\Delta E_{\text{pI–II}}$ is a consequence only of the shift of peak II since the potential of peak I remains practically the same. It is interesting to observe that the enhancement in $\Delta E_{\text{pI–II}}$ is clearly more important in the 2-nitro imidazole derivatives (more than 200 mV) than in the 5-nitro imidazole-diazole derivatives (smaller than 100 mV). Summarising, the addition of TBAI permits a better separation of the nitro radical anion signal, facilitating its analysis. In Fig. 4, we can observe the cyclic voltammograms for the six compounds with TBAI as a second supporting electrolyte. It is clear that under these conditions, the study of the nitro radical anion is feasible and in all cases it is possible to study the couple in isolation (Fig. 4, dotted lines).

From the above studies, we have found that the optimum medium for the radical anion formation was 40/60 DMF + 15 mM aqueous citrate buffer pH 9, 0.3 M KCl and 0.1 M TBAI. This medium provides reliable experimental conditions to study the electrochemical behaviour of the radicals generated from the nitro imidazole derivatives. In spite of the above, in the voltammogram of megazol, we can observe some distortion due to strong adsorption. Specifically, we can appreciate both a pre and a post peak, indicating strong adsorption of reactant and product. If we isolate the redox couple, we can observe only the pre-peak (dotted line in Fig. 4). Furthermore, from a study of the relationship of the pre-peak with the sweep rate, we have obtained voltammetric behaviour typical of a strongly adsorbed product [30], wherein the current $(I_p)_{\text{ads}}$ of the pre-peak that precedes the diffusion peak increases with the sweep rate v, while that for the diffusion peak $(I_p)_{\text{diff}}$ varies with $v^{1/2}$, consequently, $(I_p)_{\text{ads}}/(I_p)_{\text{diff}}$ increases with increasing v, as is observed in Fig. 5. The pre-peak occurs at potentials more positive than the diffusion controlled peak, because the Gibbs energy of adsorption of the product of the

reaction, i.e. the nitro radical anion from megazol, makes the reduction of megazol to the adsorbed nitro radical anion easier than to the reduction of the nitro radical anion in solution. Considering that only megazol was strongly adsorbed at the mercury electrode and by comparison of the structures of megazol and GC-284, we conclude that the presence of the sulphur atom in the diazole ring is crucial to the adsorption phenomena in the megazol molecule. Moreover, by comparison of the structures of megazol with GC-361, it is possible to conclude that the existence of a high density of electrons in the thiadiazole ring is also necessary to favour the strong adsorption.

On the other hand, in Fig. 6, we show the log–log plot between the peak current and the sweep rate for the nitro/nitro radical anion couple for all the compounds studied. From these results we can observe that in all cases the slope is about 0.5, indicating diffusion controlled behaviour.

Then, we studied the kinetic stability of the $Ar–NO_2^{\bullet-}$ species, analysing the change in the voltammetric response with the scan rate. Fig. 7 shows the effect of different sweep rates on the cyclic voltammograms of the $Ar–NO_2/Ar–NO_2^{\bullet-}$ couple of com-
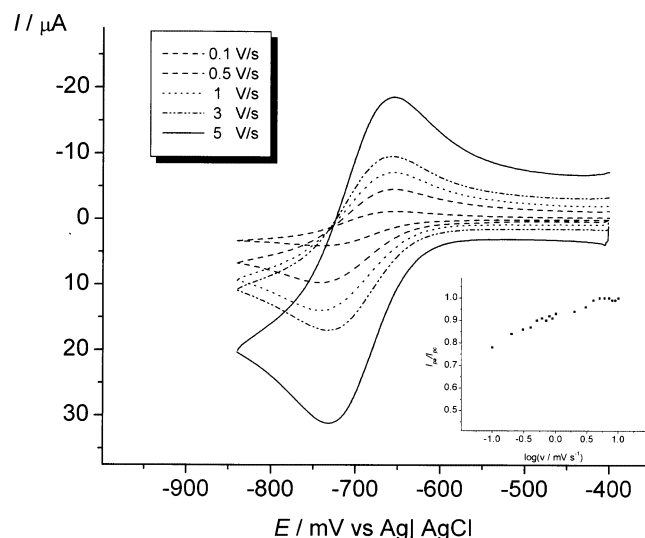

Fig. 7. Cyclic voltammograms showing the first reversible couple due to the one-electron reduction of GC-284 at different sweep rates. Inset: current ratio dependence on sweep rate for the same experiment.

pound GC-284. All the compounds show similar behaviour. As can be seen, the I_{pa}/I_{pc} current ratio
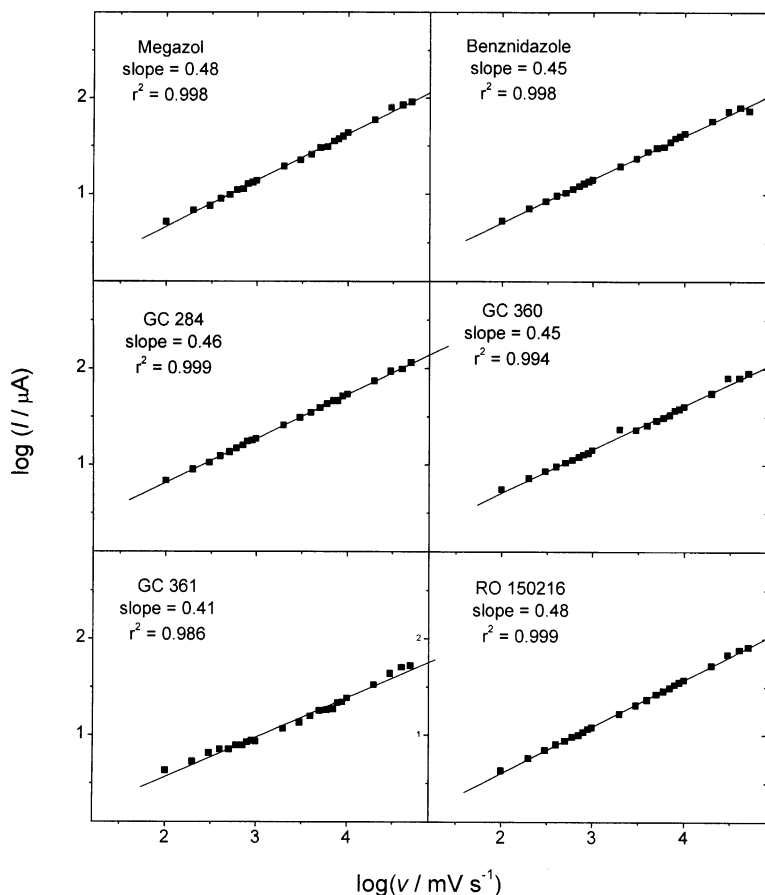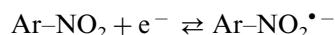

Fig. 6. Log I_p versus log v analysis for the first reduction peak of all nitrocompounds.

increases as the scan rate is increased, reaching a limiting value of one. This result fulfils the requirements for an irreversible chemical reaction following a reversible charge transfer step (EC process) [33]. We have also found that, at the same sweep rate, when the concentration of the nitro compound increases, the current ratio decreases, implying that the chemical step of the EC mechanism is a second-order reaction. In accord with our previous work [17,18] and that of other authors [34,35], the EC mechanism for these compounds can be described by (see Eq. (1)):

$$Ar\text{–}NO_2 + e^- \rightleftarrows Ar\text{–}NO_2^{\bullet-}$$

$$2Ar\text{–}NO_2^{\bullet-} + 2H^+ \rightarrow Ar\text{–}NO_2 + Ar\text{–}NO + H_2O \quad (4)$$

According to this fact we can use the theory described by Olmstead and Nicholson [29] in order to obtain the second-order rate constant of the chemical disproportionation step. This second-order rate constant will be a parameter dependent on the kinetic stability of each nitro radical anion in this medium.

The corresponding peak potential, the decay constant value (k_2) and the half-life time ($t_{1/2}$) calculated for a drug concentration of 5 mM are shown in Table 2. According to these results, it is interesting to note that the nitromidazoles (benznidazole, RO150216) are somewhat more stable than the nitroimidazole-aminothiadiazoles (megazol, GC-360 and GC-361). Probably this difference can be ascribed as a consequence of the role of the sulphur atom in the thiadiazole compounds that produces adsorption on the mercury electrode. On the other hand, Mairanovski et al. [36] reported that nitro radical anions were more stable in solution than when adsorbed on the electrode surface. Consequently, in the present case the thiadiazole compounds exhibit an enhanced adsorption producing nitro radical anions with lower kinetic stability. Moreover, if we compare megazol with the GC-284 compound, wherein the only difference is the sulphur atom in megazol as opposed to the oxygen atom in GC-284, the above assumption is

strongly validated. However, according to the similarity of the peak potential values, we can affirm that there are no significant differences in the energy requirements to form the nitro radical anion from megazol or its derivatives. On the other hand, if we compare the results with nifurtimox, the classic antichagasic drug (Table 2), there are differences in both the peak potential and k_2 values. Nifurtimox shows a lower cathodic peak potential, meaning that the radical is more easily formed, but the nitro radical anion from nifurtimox has a longer half-life showing increased kinetic stability. The same conclusion was obtained by Viodé et al. [12] by pulse radiolysis of megazol and nifurtimox as shown by the E_7^1 (peak potential at pH 7 for a one-electron transfer) and k_2 value (Table 2). These values are different from ours, because of the difference in media in the two types of experiments: a medium of low proton activity in cyclic voltammetric experiments and water in pulse radiolysis. Obviously the results in aqueous medium show that the radical anion is kinetically less stable in water.

On the other hand, recent biological studies (unpublished data), show that megazol is the most active compound in parasite growth cultures of several *T. cruzi* strains and is more active than nifurtimox. By relating the biological and electrochemical results, we can conclude that a better in vivo activity implies a larger decay constant of the radical anion, which probably means that the products of decay are more toxic to the parasite. Taking into account that the nitroso derivative is formed in the disproportionation reaction (Eq. (4)) and that it is more electrophilic than the parent nitro compound, it is reasonable to expect that the nitroso metabolite is responsible for the greater efficacy of megazol against *T. cruzi*.

3.3. Calculation of E_7^1 values

The E_7^1 values are parameters that account for the energy necessary to transfer the first electron to an

Table 2
Cathodic peak potentials, second-order decay constants and half-life times for the nitro radical anion obtained from megazol and related compounds

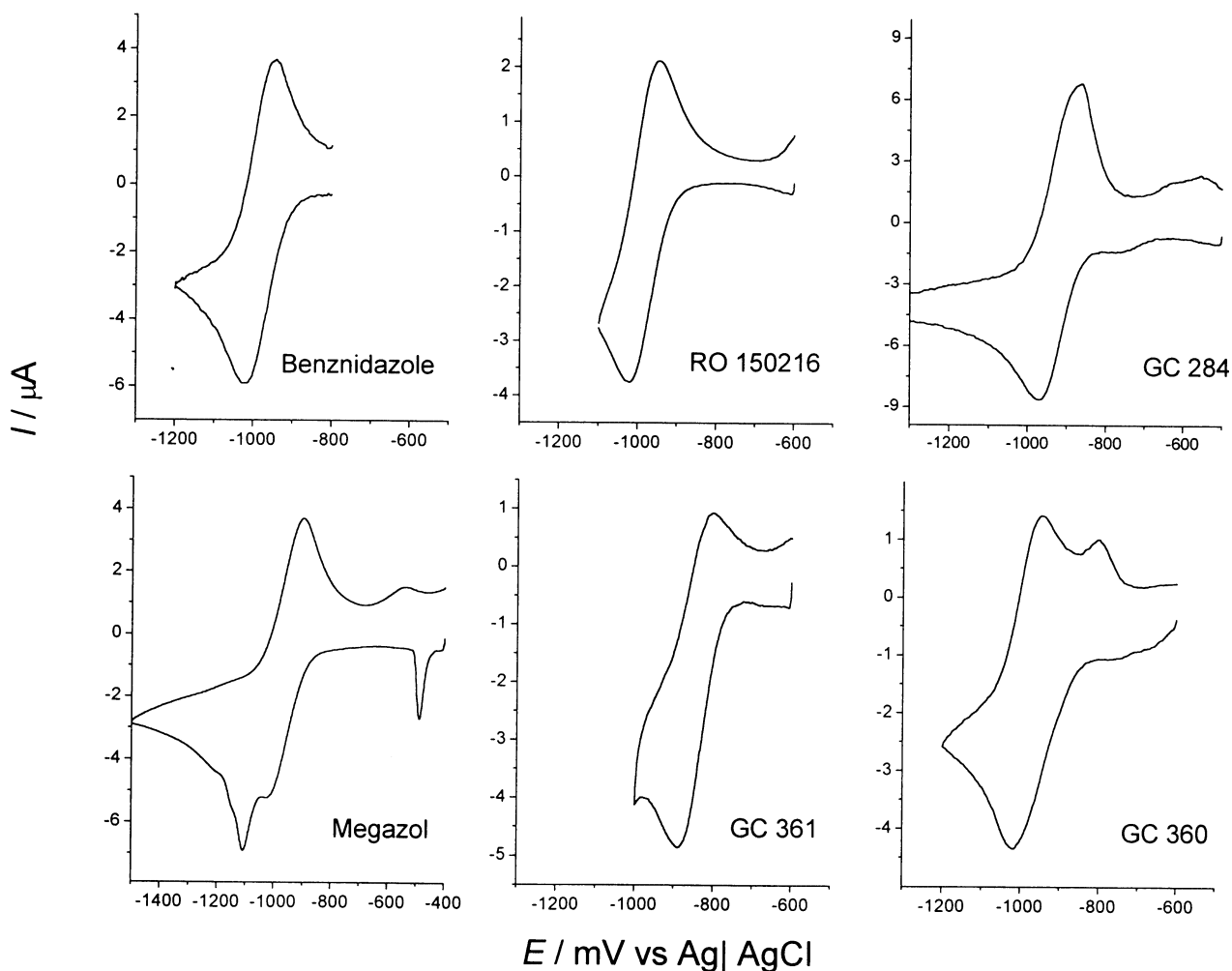|	Cyclic voltammetry			Pulse radiolysis [a]	
	E_{pc}/mV	$10^{-2}\, k_2$/M^{-1} s^{-1}	$t_{1/2}$/s	E_7^1/mV	$10^{-5}\, 2k_2$/M^{-1} s^{-1}
Megazol	−733	19	0.11	−438	7.0
GC-361	−758	33	0.06		
GC-360	−753	27	0.07		
GC-284	−742	7.8	0.26		
Benznidazole	−730	8.9	0.22		
RO 150216	−740	7.0	0.29		
Nifurtimox	−630	0.53	3.77	−260	0.1

[a] Values obtained from Ref. [12].

Fig. 8. Cyclic voltammograms of all nitrocompounds (5 mM solution) in aprotic media, DMF + 0.1 M TBAI. Sweep rate 1 V s^{-1}.

electroactive group at pH 7 in aqueous medium to form a radical anion. Therefore in the case of nitro compounds, the E_7^1 values represent the ability to form the nitro radical anion. The E_7^1 values are obtained experimentally only by electron pulse radiolysis but according to the study published by Breccia et al. [37], it is possible to obtain a correlation between the cathodic peak potentials in aprotic media with the E_7^1 values obtained with pulse radiolysis. Consequently we have obtained the cyclic voltammograms of all the compounds studied in aprotic media (100% DMF + 0.1 M TBAI). In Fig. 8 we observe the nitro/nitro radical anion couple obtained for each nitro-imidazole derivative in aprotic medium. Furthermore the E_7^1 values calculated from the E_{pc} values and comparisons with some values obtained by pulse radiolysis are shown in Table 3. From these values we can observe an adequate correlation between the E_7^1 values obtained by both cyclic voltammetry and pulse radiolysis. In the case of megazol the difference of about 50 mV can be ascribed to the strong adsorption observed in the megazol

voltammogram that would produce a little distortion in the measurement. This result is very significant because it shows that electrochemical techniques can be used to

Table 3
E_7^1 values calculated from the cathodic peak potentials in aprotic media and comparison with the values obtained experimentally by pulse radiolysis

	Cyclic voltammetry		Pulse radiolysis [a]
	E_{pc}/mV	Calculated E_7^1/mV	E_7^1/mV
Megazol	−1028	−385	−438
GC-361	−896	−253	
GC-360	−1022	−379	
GC-284	−968	−321	
Benznidazole	−1019	−376	
RO 150216	−1007	−364	
Nifurtimox	−876	−257	−260

[a] Values obtained from Ref. [12].

obtain the E_7^1 parameters for these types of compounds, thus making the determination easier and cheaper than pulse radiolysis experiments.

In conclusion, using cyclic voltammetry we were able to study the nitro radical anion formation, its kinetic stability and correlate these results with those obtained by pulse radiolysis and with the biological activity against *T. cruzi*. This will allow a fast and easy electrochemical screening of future new antichagasic compounds.

Acknowledgements

References

[1] G. Asato, G. Berkelhammer, Science 162 (1968) 1146.

[2] G. Asato, G. Berkelhammer, J. Med. Chem. 15 (1972) 1086.

[3] J. Aldunate, A .Morello, in: O.I. Aruoma (Ed.), Free Radicals in Tropical Medicine, vol. 137, Harwood Press, Switzerland, 1993, p. 165.

[4] E. Lages-Silva, L.S. Filardi, Z. Brener, Mem. Inst. Oswaldo Cruz 85 (1980) 401.

[5] L.S. Filardi, Z. Brener, Ann. Trop. Med. Parasitol. 76 (1982) 293.

[6] B. Bouteille, A. Marie-Daragon, G. Chauviere, C. de Albuquerque, B. Enanga, M.L. Darde, M. Vallat, J. Perie, M. Dumas, Acta Tropica 60 (1995) 73.

[7] F.W. Jernnings, G. Chauviere, C. Viode, M. Murray, Trop. Med. Int. Health 1 (1996) 363.

[8] B. Enanga, H. Boudra, G. Chauviere, C. Labat, B. Bouteille, M. Dumas, G. Houin, Arzneimittelforschung 49 (1999) 441.

[9] B. Enanga, J. Ndong, H. Boudra, L. Debrauwer, G. Dubreuil, B. Bouteille, G. Chauviere, C. Labat, M. Dumas, J. Périé, G. Houin, Arzneimittelforschung 50 (2000) 158.

[10] M.H. Tsuhako, M.J. Alves, W. Colli, Z. Brener, O. Augusto, Biochem. Pharmacol. 38 (1989) 4491.

[11] C. Viodé, C. De Alburquerque, G. Chauviere, C. Houee-Levin, J. Perie, New J. Chem. 21 (1997) 1331.

[12] C. Viodé, N. Bettache, N. Cenas, R.L. Krauth-Siegel, G. Chauviere, N. Bakalara, J. Périé, Biochem. Pharmacol. 57 (1999) 549.

[13] D.I. Edwards, R.C. Knight, A. Zahoor, Int. J. Radiat. Oncol. Biol. Phys. 12 (1986) 1207.

[14] J.H. Tocher, D.I. Edwards, Biochem. Pharmacol. 48 (1994) 1089.

[15] D. Barety, B. Resibois, G. Vergoten, Y. Moschetto, J. Electroanal. Chem. 162 (1984) 335.

[16] L.J. Núñez-Vergara, J. Aldunate, M.E. Letelier, S. Bollo, Y. Repetto, A. Morello, P.L. Spencer, J.A. Squella, Bioelectrochem. Bioenerg. 38 (1995) 355.

[17] L.J. Núñez-Vergara, S. Bollo, A.F. Alvarez, M. Blázquez, J.A. Squella, J. Electroanal. Chem. 345 (1993) 121.

[18] L.J. Núñez-Vergara, F. Garcia, M. Dominguez, J. de la Fuente, J.A. Squella, J. Electroanal. Chem. 381 (1995) 215.

[19] L.J. Nuñez-Vergara, P.A. Naverrete-Encina, M.E. Ortiz, S. Bollo, J.A. Squella, Chemico-Biol. Interact. 101 (1996) 89.

[20] L.J. Núñez-Vergara, C. Matus, A. Alvarez, B.K. Cassels, J.A. Squella, Electroanalysis 6 (1994) 509.

[21] L.J. Núñez-Vergara, D. Farias, S. Bollo, J.A. Squella, Bioelectrochem. Bioenerg. 53 (2001) 103.

[22] L.J. Núñez-Vergara, J.C. Sturm, C. Olea-Azar, P. Navarrete-Encina, S. Bollo, J.A. Squella, Free Radical Res. 32 (2000) 399.

[23] J.A. Squella, P. Gonzalez, S. Bollo, L.J. Núñez-Vergara, Pharmaceut. Res. 16 (1999) 161.

[24] S. Bollo, L.J. Núñez-Vergara, M. Bontá, G. Chauviere, J. Périé, J.A. Squella, Electroanalysis 13 (2001), in press.

[25] G. Chauviere, C. Viodé, J. Perie, J. Heterocyclic Chem. 37 (2000) 119.

[26] A.G. Gonzalez, F. Pablos, A. Asuero, Talanta 39 (1992) 91.

[27] A. Asuero, M.A. Herrador, G. Gonzalez, Talanta 40 (1993) 479.

[28] R.S. Nicholson, Anal. Chem. 36 (1964) 1406.

[29] M.L. Olmstead, R.S. Nicholson, Anal. Chem. 41 (1969) 862.

[30] R.H. Wopschall, I. Shain, Anal. Chem. 39 (1967) 1514.

[31] S. Bollo, PhD Thesis, University of Chile, 1997.

[32] H. Lund, in: H. Lund, M. Baizer (Eds.), Organic Electrochemistry, Marcel Dekker, New York, 3rd edn., 1990, Ch. VI, p. 307.

[33] R.S. Nicholson, Anal. Chem. 36 (1964) 706.

[34] J.H. Tocher, D.I. Edwards, Free Radical Res. Commun. 16 (1992) 19.

[35] A. Radi, F. Belal, J. Electroanal. Chem 441 (1998) 39.

[36] S. Mairanovskii, J. Stradins, I. Kravis, Sov. Electrochem. 8 (1972) 766.

[37] A. Breccia, G. Berrilli, S. Bofia, Int. J. Radiat. Biol. 36 (1979) 85.