Recent Developments in the Electrochemistry of Some Nitro Compounds of Biological Significance

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Abstract: The redox chemistry of different nitro compounds of biological significance is focused to understand how the reduction of the nitro group can play an active role in several aspects as: electroanalytical determinations, free radical generation and stability and free radical reactivity. We have focused our studies to a lot of pharmaceuticals belonging mainly to the following families: calcium antagonists as nitrobenzene substituted 1,4-dihydropyridines, antibacterial and anti protozoan agents as nitroimidazoles and nitrofurans.

The formation of the nitro radical anion as the product of the one electron reduction of nitro compounds generates a series of important consequences passing from chemical to biological aspects. We have used electrochemical techniques to study the formation, stability and reactivity of this nitro radical anion in different media. From cyclic voltammetric experiments it is possible qualitatively to visualize the formation of the nitro radical anion through the one-electron reversible couple due to the redox system nitro / nitro radical anion . Furthermore also is possible the quantitative determination of the kinetic rate constant of the nitro radical anion decay and the interaction constants with other molecules. Although substituents may affect the redox potential and consequently the stability or reactivity of the nitro radical anions, other factors are important in regulation of these properties. Among these factors, it is possible to mention the nature of the reaction media making possible the occurrence of intermolecular reactions of the father-son type between the nitro radical anion and an acidic hydrogen present in the molecules.

INTRODUCTION

The study of the electrochemistry of nitro compounds began early in 1900, when Haber [1] revealed the stepwise reduction of nitrobenzene, but there is still a great interest in these type of compounds. One reason for such interest is that numerous nitro compounds are manufactured for use as pharmaceuticals and consequently they are introduced into living organisms and metabolized often via redox type processes. The electrochemistry of these nitro compounds has been reviewed by several authors [2-5] and it is possible to conclude that the reduction involves, in general terms, a common mechanism with a series of one-electron additions and chemical steps wherein the differences are in the chemical follow up reactions rather than in the electro-chemical steps. In fact, the electrochemical reduction mechanisms of aromatic and heterocyclic nitro compounds have been profusely investigated by organic electrochemists. Those studies reveal that the mechanisms are very complex and strongly dependent on the nature of the reaction medium. From our point of view the better electrochemical technique to study the reduction of nitro compound is the cyclic voltammetry. In fact, in aqueous medium [6-11], in the absence of inhibitor substances, only one irreversible cyclic voltammetric peak (i.e. peak I_c in Fig. (**1**)) corresponding to the four-electron, four-proton nitro compound reduction to the hydroxylamine derivative (eq. **1**) is obtained:

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

At higher potentials, in acidic medium (generally between pH 2-5), appears another irreversible peak (i.e. peak II_c in Fig. (**1**)) corresponding to the further reduction of the protonated hydroxylamine derivative to produce the amine derivative according to the following overall reaction:

$$RNHOH_2^+ + 2e^- + H^+ \rightarrow RNH_2 + H_2O \quad (2)$$

Furthermore in the positive potential direction sweep it is possible to appreciate at, more positive potentials, an anodic peak (i.e. peak I_a in Fig. (**1**)) corresponding to the oxidation of the hydroxylamine derivative to produce the nitroso derivative. Moreover in a second cycling it is possible to observe the corresponding cathodic peak due to the reduction of the nitroso derivative. These two last peaks forming a quasi reversible couple according to the following equation:

$$RNHOH \rightleftharpoons RNO + 2e^- + 2H^+ \quad (3)$$

In the Fig. (**1**) we can observe typical cyclic voltammograms wherein all the above peaks and steps are shown.

On the other hand, in alkaline solutions or in the presence of inhibitors [7-9], or by adding aprotic co-solvents [12-15], the behaviour is rather different. In fact, instead of the irreversible four-electron reduction peak, two different signals appear. First, a reversible couple (i.e. peaks I'_c and I'_a

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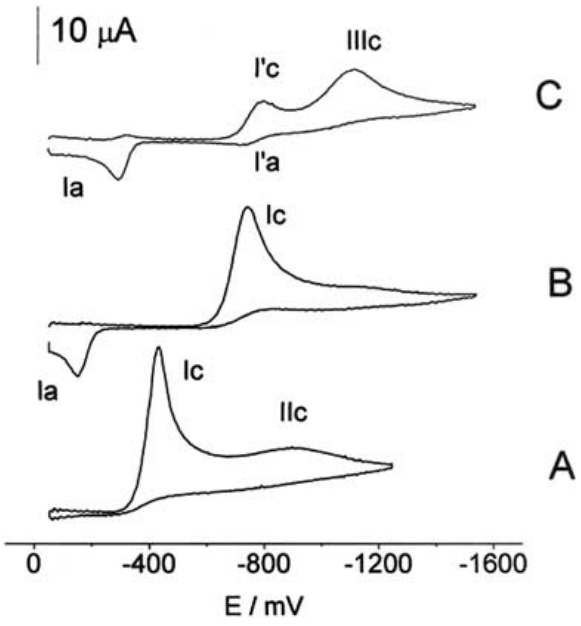


Fig. (1). Typical cyclic voltammogram of nitroaromatic compounds in different media: A= protic acid medium, B= Protic medium and C= protic alkaline or in presence of inhibitors, or also adding aprotic co-solvents.

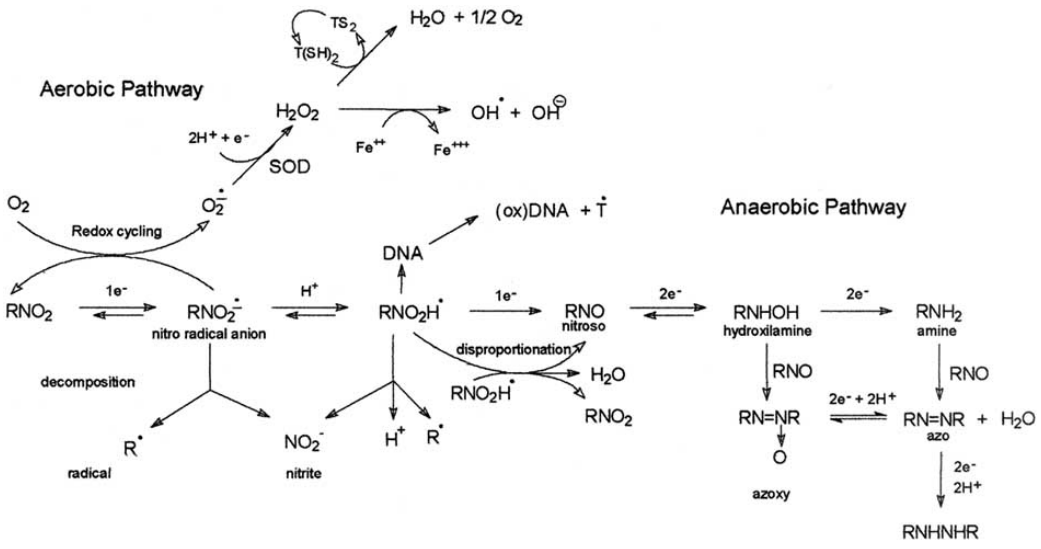
in Fig. (**1**) due to the one-electron reduction of the nitro group to form the corresponding nitro radical anion ($RNO_2^{\cdot-}$) and second an irreversible peak (i.e. peak III_c in Fig.(**1**)) due to the further reduction of the nitro radical anion to form the corresponding hydroxylamine derivative according to the following equations:

$$RNO_2 + e^- \rightleftharpoons RNO_2^{\cdot-} \qquad (4)$$

$$RNO_2^{\cdot-} + 3e^- + 4H^+ \rightarrow RNHOH + H_2O \qquad (5)$$

From the biological point of view the reductive activation presents an elegant way of targeting drugs to cells with a limited oxygen contents. The compounds with the most extensive clinical application in this capacity are the nitroaromatics that have been employed in the treatment of anaerobic infections and in cancer treatment as hypoxic cell cytotoxins and radiation sensitizers. Structures examined include from the heterocyclic compounds as imidazoles and furans to the benzenoids and polycyclic aromatics [16]. All the biological activity of the nitrocompounds depends on the reduction of the nitro group, which can accept up to 6 electrons to give the amine derivative [17]. The bioreductive pathway for nitroaromatic compounds is displayed in scheme (**1**). From this scheme it can be emphasized that only organisms that have redox mechanisms with a sufficient potential to reduce the nitro group, will produce the biologically active form of the compound. Aerobic organisms generate the well-known "futile-cycle" reaction whereby O_2 accepts one electron from the $RNO_2^{\cdot-}$, regenerating the original bioactive compound and forming superoxide ($O_2^{\cdot-}$). Although a detailed understanding of the mechanisms involving the biological activity of nitro compounds is still a matter of debate, there is no question that the use of these compounds in medicine and cancer therapy relies upon free radical mechanisms. Specifically the redox properties of the one-electron couple RNO_2 / $RNO_2^{\cdot-}$ define virtually all the biological properties of these compounds [18].

As reported above, the biological activity of some nitroaromatic compounds is due to the redox chemistry of the nitro group. This factor has motivated a great number of electrochemical studies and consequently, in this review, the redox chemistry of different nitro compounds of biological significance is focused to understand how the reduction of the nitro group can play an active role in several aspects as: electroanalytical determinations, free radical generation, stability and reactivity.



Scheme 1.

ELECTROANALYTICAL DETERMINATIONS

Since polarography was applied to pharmaceutical analysis relatively early in the development of the method, there has been a great advance in this field. Several recent reviews have been published on the topic [19-22]. The main basic requisite to develop an electrochemical determination of a pharmaceutical compound is the existence of an electroactive group or a group capable to be quantitatively converted in an electroactive one. In the case of the nitro pharmaceutical compounds the most useful signal for quantitative purposes is the four-electron four-proton signal due to the reduction of the nitro group to produce the hydroxylamine derivative as shown in eq. (**1**). The advantages of this signal are both the high quantity of current produced per mol of electroactive compound and the relatively low energy requirements for the nitro reduction. Consequently we can obtain high current / concentration ratios and low reduction potentials. These advantages have permitted the development of a great quantity of electrochemical methods applied to nitro compounds of biological significance but in this review we only will describe the survey of this topic in the last decade. In Table (**1**) we have summarized a series of analytical applications of some nitro compounds of biological significance by electrochemical techniques. The commonly used electrochemical techniques for determination of nitro compounds of biological significance are: tast polarography (TP), differential pulse polarography (DPP), linear sweep voltammetry (LSV), cyclic voltammetry (CV), hydrodynamic voltammetry (HV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and several stripping voltammetry techniques such as cathodic stripping voltammetry (CSV), adsorptive stripping voltammetry (AdSV), adsorptive cathodic stripping voltammetry (AdCSV), square wave adsorptive stripping voltammetry (SWAdSV), differential pulse adsorptive

Table 1. Analytical determination of some nitro compounds of biological significance by electrochemical techniques

Compound	Working electrode	Detection or quantification limits (order)	Used technique	Reference
Metronidazole	GCE (activated)	10^{-6} M	DPV	26
	Coated GCE	10^{-9} M	DPV	27
	Hg	10^{-7} M	DPP	28
Secnidazole	Hg	10^{-5} M	DC & AC Polarog.	29
	Hg	10^{-7} M	DPP	30
	Hg	10^{-9} M	AdSV	31
Benznidazole	Modified GCE	-	DPV	32
	Hg	-	DPP	33
Tinidazole	Hg	10^{-7} M	DPP	35
	GCE (activated)	10^{-6} M	LSV	36
Ornidazole	GCE (activated)	10^{-6} M	LSV	37
Megazol	Hg	10^{-6} M	DPP	38
PA-824	Hg	10^{-7} M	DPP	39
	GCE	10^{-7} M	DPV	39
Furazolidone	HMDE	10^{-9} M	AdCSV	40
Furaltadone	HMDE	10^{-9} M	AdCSV	40
Nitrofurazone	HMDE	10^{-9} M	AdSV	41
Nitrofurantoin	HMDE	10^{-9} M	CSV	42
	HMDE	10^{-10} M	SWAdSV	43
Nifuroxime	Modified GCE	21 ng	SISA	45
Nifuroxazide	Hg	10^{-7} M	DPP	46
	HMDE	10^{-9} M	ASV	47
	Hg, CPE	10 ng/ml	ASV	48
Nifedipine	GCE (activated)	10^{-6} M	LSV	49
	HMDE	10^{-9} M	SWAdSV	50
	HMDE	10^{-10} M	SWAdSV	51
	CPE (modified)	10^{-10} M	DPAdSV	52
Nimodipine	CPE (modified)	10^{-10} M	DPAdSV	52
Nitrendipine	GCE	10^{-6} M	LSV	53

Table 1. Analytical determination of some nitro compounds of biological significance by electrochemical techniques. (Continue)

Compound	Working electrode	Detection or quantification limits (order)	Used technique	Reference
Nitrendipine	GCE	10^{-6} M	LSV	53
Nicardipine	GCE (modified)	10^{-7} M	LSV	54
	Hg	10^{-5} M	DPP	55
Nisoldipine	Hg, GCE	10^{-6} M	DPP, DPV	56
	GCE	10^{-6} M	DPV	57
Lercanidipine	Hg	10^{-6} M	DPP	58
Nimesulide	Hg	10^{-6} M	DPP	59
Ranitidine	Hg coated Pt	10^{-8} M	SWV	60
Loratadine	Hg	10^{-5} M	DPP	62
Propranolol	HMDE	2-5 ng/ml	AdSV	63
Praziquantel	HMDE	10^{-8} M	AdSV	64

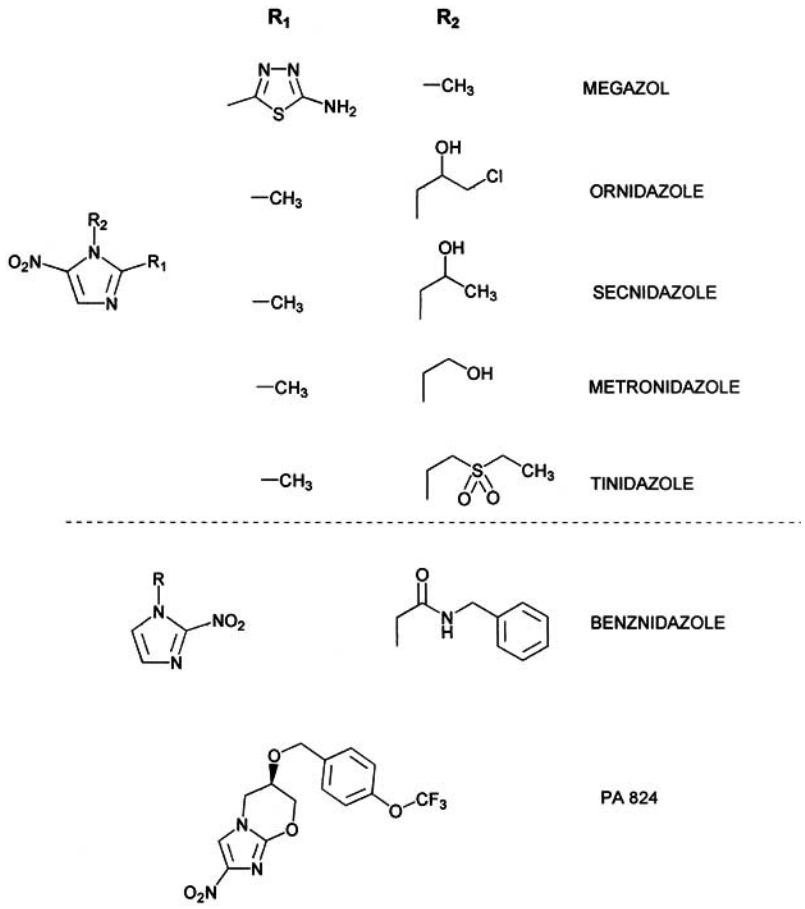


Fig. (2). Molecular structures of some selected nitroimidazoles of biological significance.

stripping voltammetry (DPAdSV) etc., which involve a pre concentration step followed by voltammetric and polarographic measurements.

One of the most important families of nitro compounds of biological significance are the nitroimidazole derivatives. The molecular structure of some of these members are shown in Fig. (**2**). In the last few decades, nitroimidazoles

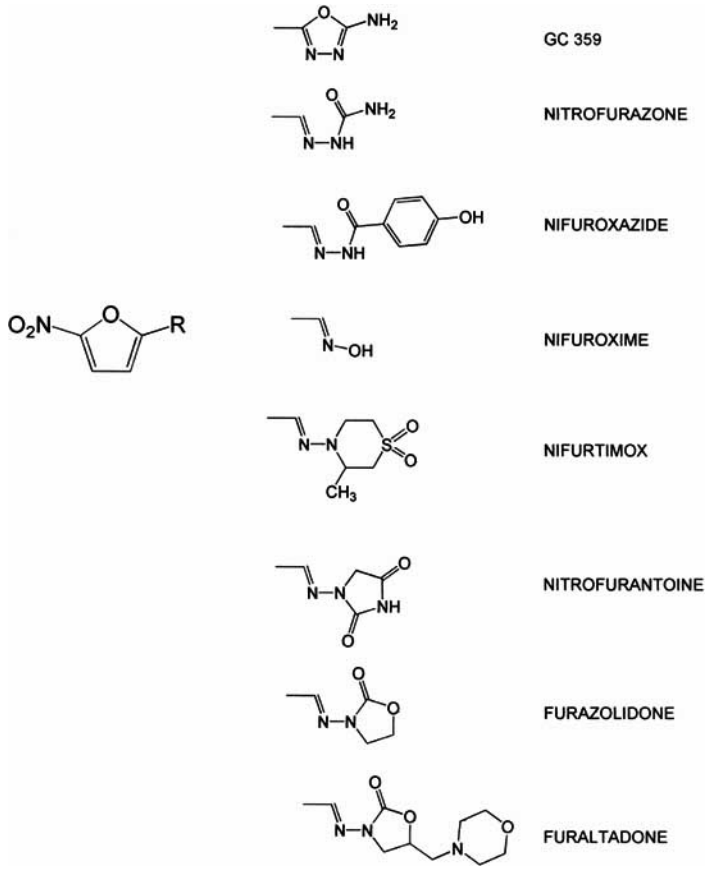


Fig. (3). Molecular structures of some selected nitrofuran derivatives of biological significance.

have been the source of many investigations due to their properties as antibiotics, radiosensitizers and anti-protozoans [23,24]. Metronidazole is the most important drug of the group of 5-nitroimidazoles and several electrochemical studies have been described for its determination. The voltammetric behavior of nitroimidazole on a DNA-biosensor with the objective to detect *in-situ* the damage caused to DNA on the electrode surface and the voltammetric reduction of metronidazole at activated glassy carbon electrode (GCE) and its determination in pharmaceutical dosage forms have been described [25,26]. More recently, the electrochemical reduction and voltammetric determination of metronidazole at a nanomaterial thin film coated GCE was described [27]. Compared with bare GCE this modified GCE significantly enhances the reduction peak current of metronidazole. Furthermore a polarographic determination of metronidazole with a detection limit of 1×10^{-7}M has been proposed [28]. The determination of secnidazole in pharmaceutical tablets by DC and AC polarography [29] , in tablets and intestinal fluids by DPP [30] and in urine by AdSV has been published [31]. Another nitroimidazole used commonly as a therapeutic agent against Chagas's disease is benzdinazole which has been voltammetrically determined in a DNA-electrochemical biosensor [32]. The DNA-biosensor enabled pre-concentration of the drug onto the electrode surface and the *in-situ* detection of the damage caused to the DNA immobilized on the electrode surface. The polarographic determination of benznidazole has also been carried out in non-aqueous medium [33] and its electrochemical behaviour was studied by cyclic voltammetry and controlled potential electrolysis [34]. The determination of tinidazole in tablets has been carried out by a DPP method in aqueous solution with a recovery of 98.7 % with a 3% of RSD [35]. A simple and fast method for the determination of tinidazole in tablets using a GCE activated by applying a new pre-treatment was described [36]. The same activated GCE was applied to the voltammetric determination of ornidazole in pharmaceutical dosage forms [37]. Megazol, a highly active compound used against several strains of *Trypanosoma cruzi*, the causative agent of Chagas disease, was electrochemically studied and a DPP method was proposed [38]. The voltammetric determination of a method for the determination of a nitroimidazopyran (PA-824) drug candidate for the treatment of tuberculosis by DPP and DPV at pH 7 has been recently reported [39].

Other important family of nitro compounds with biological significance are the nitrofuran derivatives which have been extensively used as antibacterial agents (Fig. (**3**)). Adsorptive cathodic stripping voltammetry (AdCSV) was

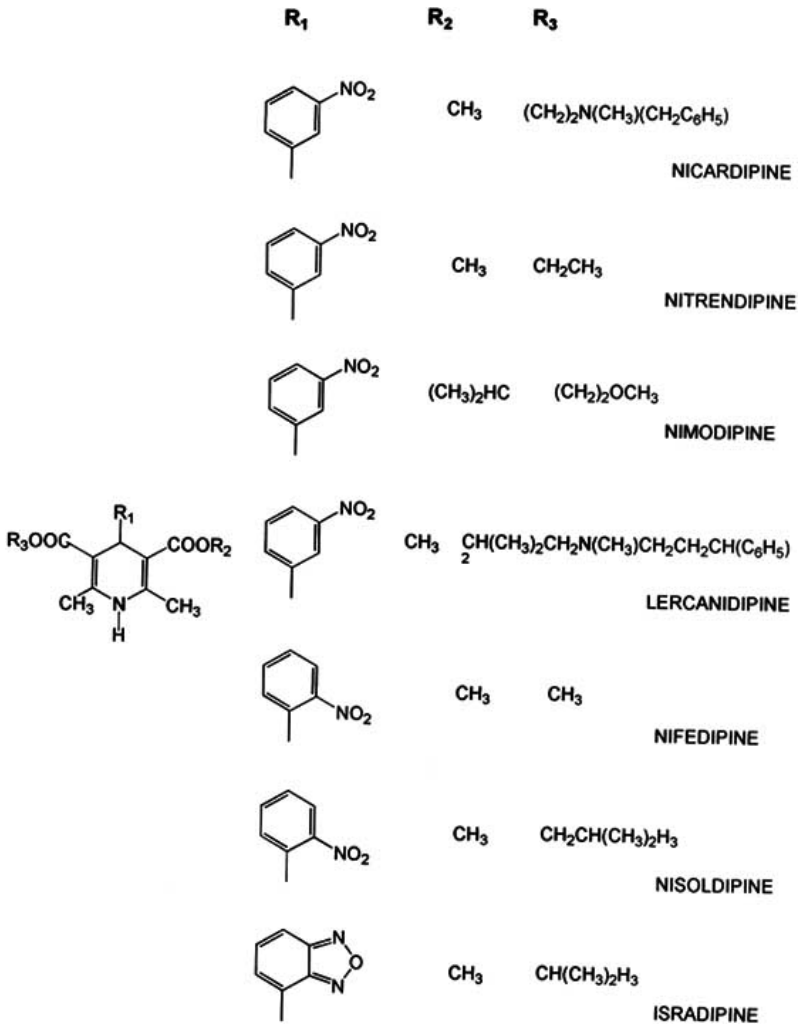


Fig. (4). Molecular structures of some selected nitrobenzene substituted 1,4-dihydropyridine derivatives of biological significance.

used for the determination of furazolidone, furaltadone and nitrofurazone in pharmaceutical formulations, urine and serum samples using HMDE as working electrode [40,41]. The antibacterial drug nitrofurantoin was determined by CSV using the adsorptive peak at -0.173 V, pH 5.5 [42]. Recently a very sensitive method for the determination of nitrofurantoin in pharmaceutical formulations and biological fluids by square-wave cathodic adsorptive stripping voltammetry has been revealed [43]. Also recently nitrofurantoin has been determined using voltammetry at activated carbon fiber microelectrodes. This methodology was also used to carry out degradation studies of nitrofurantoin in acidic and basic media under photodegradation or thermal degradation conditions [44]. The voltammetric behavior of nifuroxime was investigated comparing stationary voltammetric methods with the recently proposed sequential-injection stripping analysis (SISA), by using cyclic voltammetry (CV) and differential-pulse voltammetry at bare and DNA-modified glassy carbon (GC) electrodes [45]. The determination of urinary tract antibiotic nifuroxazide in capsules was investigated by DPP [46] showing adequate precision and accuracy reaching a detection limit of 1×10^{-7} M. Also the determination of nifuroxazide in human serum by adsorptive stripping voltammetry [47] after adsorptive accumulation on the surface of HMDE has been developed. Furthermore the determination of nifuroxazide with polarography and ASV at mercury and carbon paste electrodes was reported [48]. In the latter, modification of the carbon paste by addition of nonpolar polystyrene/divinylbenzene particles was investigated to enhance the adsorption properties of the surface. Concentrations as low as 10 ng/mL could be determined in urine without interferences.

1,4-dihydropyridines (1,4-DHP) from the nifedipine's family Fig. (**4**) is a very common group of drugs with antihypertensive properties. This type of compounds have a 4-nitrobenzene substituent on the DHP ring given it electroreductive properties, but in this case, the nitro group is

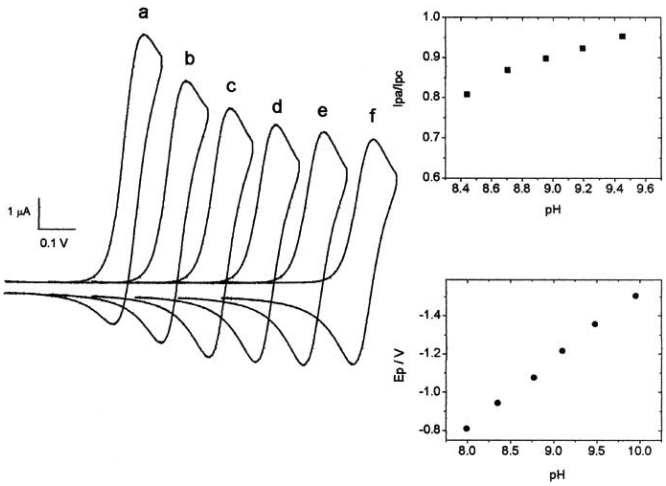


Fig. (5). Cyclic voltammograms for the couple $RNO_2/RNO_2^{\cdot-}$ at different pH with the corresponding E_P vs pH and $i_{p,a}/i_{p,c}$ vs pH plots in the inserts.

not essential for its pharmacological action. The voltammetric determination of nifedipine based on the reduction of the drug at a glassy carbon electrode activated by applying a new pre-treatment has been reported [49]. A simple, sensitive and selective square-wave adsorptive stripping voltammetric method has been developed and validated for the determination of nifedipine in plasma [50]. More recently, a fully validated sensitive cathodic adsorptive stripping square-wave voltammetry procedure was optimized for the determination of nifedipine at trace levels in bulk, pharmaceutical formulation and human serum [51]. A simple, rapid, and selective differential pulse adsorptive stripping voltammetric (DPAdSV) method has been developed for the determination of nifedipine and nimodipine at a bare carbon-paste electrode (CPE) and a clay-modified carbon-paste electrode (CMCPE). The method was applied successfully for the direct determination of nifedipine and nimodipine in tablet dosage forms, urine, and serum samples [52]. The voltammetric behavior of nitrendipine and its application to the direct determination of nitrendipine in tablets and urine has been proposed on glassy carbon electrode [53]. The direct voltammetric determination of nicardipine hydrochloride in tablets at a Cobalt/GC ion implantation modified electrode and also a pulse polarographic determination have been revealed [54,55]. The electroanalytical determination of nisoldipine has been developed using both anodic and cathodic responses. The analytical methods were successfully applied to the determination of nisoldipine in both tablets and capsules [56,57]. Lercanidipine is a new third generation 1,4-dihydropyridine calcium antagonist derivative used in hypertension treatments. A DPP method was successfully applied to the individual tablet assay in order to verify the uniformity content of lercanidipine in commercial tablets [58]. Other miscellaneous nitro drugs which can be determined by electroanalytical techniques are nimesulide [59] and ranitidine [60].

In the case of molecules lacking of a nitro group the use of nitration as a method of derivatising aromatic species such that the resulting nitro functionality can serve as an electroactive label has been examined [61]. Loratadine, a potent antihistamine drug, is not directly electroreducible at a dropping mercury electrode; however, by means of a nitration procedure it is possible to obtain a nitro-loratadine derivative. The derivative exhibits a DPP peak due to the reduction of the nitro group. This peak was used in order to develop an analytical procedure for determining loratadine in pharmaceutical dosage forms [62]. The indirect determination of propranolol after derivatization with nitric acid by cathodic adsorptive stripping voltammetry has been carried out in human urine and serum [63]. Also the indirect determination of praziquantel in human serum by cathodic adsorptive stripping voltammetry after nitration of the parent drug has been reported [64].

NITRO RADICAL ANION GENERATION AND STABILIZATION

As can be seen from eq. (4) and scheme (**1**) the monoelectronic transfer of the nitro compounds to produce the corresponding $RNO_2^{\cdot-}$ is the first and mandatory first step in the reduction pathway of the nitro compounds. The prototropic properties and natural lifetimes of $RNO_2^{\cdot-}$ have been studied mainly by using electron spin resonance (ESR) and pulse radiolysis techniques [65-67] but lately electrochemical techniques i.e. cyclic voltammetry, have also shown to play an advantageous role in the study of $RNO_2^{\cdot-}$. 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, $RNO_2/RNO_2^{\cdot-}$. In figures (**5**) and (**6**) we can appreciate cyclic voltammograms corresponding to the $RNO_2/RNO_2^{\cdot-}$ couple at both different sweep rates and different conditions of the medium. By using appropriately the wide versatility of the cyclic voltammetric technique, it is possible to study the generation of the $RNO_2^{\cdot-}$ and their stability in different media. The generation of the $RNO_2^{\cdot-}$ depends fundamentally from the energy requirements to introduce one electron at the nitro

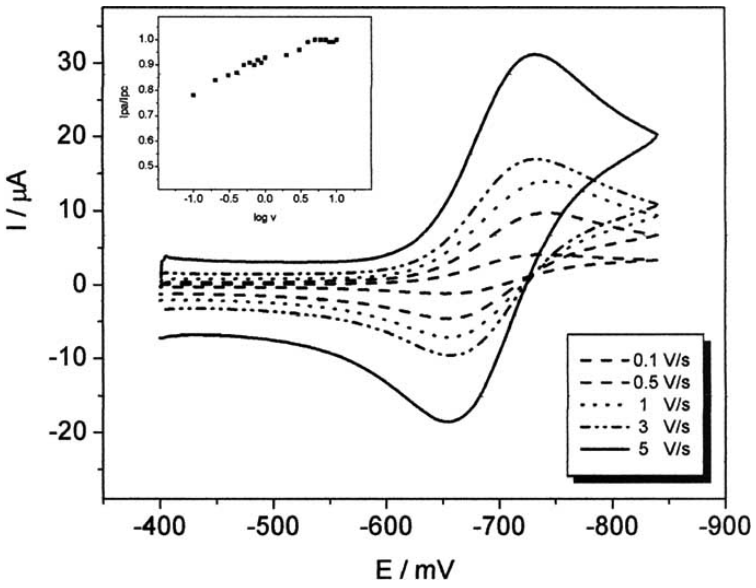


Fig. (6). Cyclic voltammograms for the couple $RNO_2/RNO_2^{\cdot-}$ at different sweep rates with the corresponding $i_{p,a}/i_{p,c}$ vs log sweep rate plot in the insert. (Data from ref. 80 with permission from Elsevier).

moiety and is reflected in his half wave potentials, $E_{1/2}$. The stability of the $RNO_2^{\cdot-}$ depends fundamentally of the reaction media and is reflected in the decay of the corresponding oxidation current, $i_{p,a}$, subsequent to its electrochemical generation. In fact the stability of the nitro radical anion is better expressed by the current ratio parameter, $i_{p,a}/i_{p,c}$, which reveals the tendency of an electrochemical generated species, i.e. nitro radical anion, to undergo chemical following reactions [68]. Thus the current ratio equals to unity in the absence of further reactions of $RNO_2^{\cdot-}$ but decreases if the radical reacts subsequently. Therefore, the cyclic voltammetric experiment can be used to prove the stability of the $RNO_2^{\cdot-}$ species by changing electrochemical and chemical conditions, and then by measuring the $i_{p,a}/i_{p,c}$ values of the $RNO_2/RNO_2^{\cdot-}$. Consequently, the $i_{p,a}/i_{p,c}$ ratio is a fundamental parameter in the characterization of $RNO_2^{\cdot-}$ and preferentially must be obtained using the Nicholson's procedure [69]. Summarizing, the $RNO_2^{\cdot-}$ generation and its stability can be represented by an EC mechanism wherein a first electrochemical step is followed by a coupled chemical reaction according to the following equations:

$$RNO_2 + e^- \rightleftharpoons RNO_2^{\cdot-} \qquad (6)$$

$$m\ RNO_2^{\cdot-} \rightarrow \text{Products} \qquad (7)$$

The chemical coupled reaction could be of first (m=1) or higher (m≠1) order depending of the type of nitro compound and the nature of the medium. A diagnostic criterion suitable for discriminating EC processes involving chemical coupled reactions of first order from those of second order is given by the dependence of $i_{p,a}/i_{p,c}$ value on the concentration of RNO_2 which is observed only in the latter case [70]. The normal decay pathway of most $RNO_2^{\cdot-}$ is that of second order being disproportionation or dimerization reactions. The second order disproportionation reaction can be described by the following equations:

$$2\ RNO_2^{\cdot-} + 2H^+ \rightarrow RNO_2 + RNO + H_2O \qquad (8)$$

but in presence of protons the protonated nitro radical anion ($RNO_2H^{\cdot}$) also can exist producing analogous disproportionation reactions according to the following equations [65]:

$$RNO_2^{\cdot-} + RNO_2H^{\cdot} \rightarrow RNO_2 + RNO + OH^- \qquad (9)$$

$$RNO_2H^{\cdot} + RNO_2H^{\cdot} \rightarrow RNO_2 + RNO + H_2O \qquad (10)$$

The cyclic voltammetric technique provides a very adequate procedure to evaluate the kinetic constants of the second order chemical reactions. Olmstead *et al.* described a theory of cyclic voltammetry for both a dimerization or disproportionation reactions initiated electrochemically [71-73]. In figure (**7**) we can observe theoretical working curves obtained for the developed theory for dimerization and disproportionation, respectively. Using these theoretical approaches, the $i_{p,a}/i_{p,c}$ values experimentally measured at each scan rate are interpolated into the working curve to determine the parameter ω, which incorporates the effects of the rate constant, nitro compound concentration and scan rate. A plot of ω versus τ resulted in a linear relation described by $\omega = k_2 C^o \tau$ (for $a\tau = 4$) where k_2 is the second order rate constant for the decomposition of $RNO_2^{\cdot-}$, C^o is the nitro compound concentration and $\tau = (E_\lambda - E_{1/2})/\nu$. Consequently, the second order rate constant for the decomposition of the nitro radical anion can be obtained from the slope of the straight line ω versus τ.

Several nitro compounds of biological significance are capable to electrochemically generate $RNO_2^{\cdot-}$ specie and its stability have been studied using the cyclic voltammetric

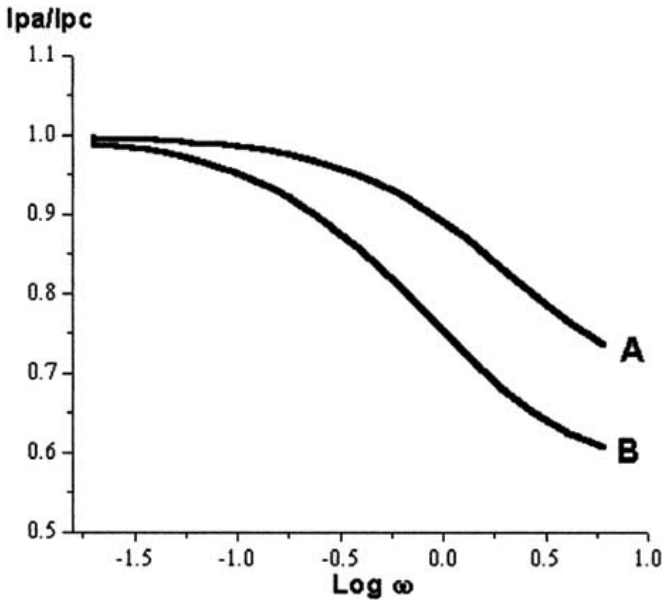


Fig. (7). Theoretical curves according to the theory developed by Olmstead *et al.*, for disproportionation (A) and dimerization (B) rate constants. (Data from ref. 71 with permission of the American Chemical Society).

approach. In Table **2** we have summarized a series of representative published results for calculated second order rate constants of several nitro compounds of biological significance. Nitroimidazole derivatives are an important family of nitro compounds whose biological action mechanism is totally dependent of its nitro radical anion derivative formation, consequently there are a permanent interest for studying this type of compounds. One of the first studies, about the influence of solvent on the electroreduction of some nitrocompound was carried out by Tocher *et al.* [1]. In this paper they compared the stability of radicals anions formed from misonidazole and metronidazole analizing the current ratio of the RNO_2 / $RNO_2^{\cdot-}$ couple of each radical obtained below the same conditions but they did not calculate kinetic constants for the degradation of the radical. Following the work of Tocher *et al.* an electrochemical study on metronidazole using aqueous/DMF solvents was conducted [75]. In this work the disproportionation rate constant, k_2, value for different quantities of organic co-solvent was calculated. Furthermore an extrapolated value at 0% DMF, simulating the value in aqueous media, was obtained. The comparison of electrochemically obtained, k_2, values [76] for metronidazole with other previously obtained by pulse radiolysis [77] in the same conditions were performed. For pulse radiolysis the result was a k_2 of 7.9×10^4 $M^{-1}s^{-1}$ which is very similar to 8.4×10^4 $M^{-1}s^{-1}$ obtained by cyclic voltammetry showing a very reasonable agreement between both techniques. Other study with the mercury electrode in 50 % DMF - citrate buffer pH 7.4 showing a value of 6.6×10^3 $M^{-1}s^{-1}$ for k_2 was also informed [78]. More recently [79] cyclic voltammetry was used to investigate the electrochemical reduction of metronidazole at glassy carbon and gold electrodes at different pHs in aqueous solution as well as in mixed medium. Also recently the stability of the nitro radical anion from metronidazole in comparison with the free radical formed from a new antituberculosis agent as PA-824 was informed [80]. On the other hand, the nitro radical anion from megazol, a new antichagasic drug, and some related nitroimidazoles was studied in aprotic medium [81] and in mixed medium [82]. The corresponding $RNO_2^{\cdot-}$ decays by a dimerization reaction in aprotic medium instead of the typical disproportionation observed in mixed medium. Furthermore the k_2 obtained values for megazol were compared with those values of the corresponding $RNO_2^{\cdot-}$ obtained from nifurtimox and benznidazole, the classic antichagasic drugs [82]. The results revealed that cyclic voltammetry is a good alternative to the classic pulse radiolysis method to obtain reliable values of the E^1_7 (thermodynamic reversible potential for the couple nitro / nitro radical anion in aqueous medium at pH 7) one-electron parameter for nitro radical anions. The electrochemical behavior of 2-(5-amino-1,3,4-oxadiazolyl)-5-nitrofuran (NF-359) and its comparison with the well-known drugs such as nifurtimox (NFX) and nitrofurazone (NFZ) in protic, mixed and aprotic media was studied [83]. The decay constants of the corresponding $RNO_2^{\cdot-}$ in mixed and aprotic media were obtained. In mixed medium, data fit well with a disproportionation reaction of the $RNO_2^{\cdot-}$ but in aprotic medium fit better with a dimerization reaction. Cyclic voltammetry and controlled potential electrolysis were used to generate the $RNO_2^{\cdot-}$ from nitrofurantoin and the second order decay constant was determined in both mixed and aprotic media [84]. The generation of the $RNO_2^{\cdot-}$ from nifuroxazide was studied and the obtained stability constants and half life times for the decay of the free radical were compared with other nitrofuran drugs such as nifurtimox, nitrofurazone and furazolidone [85].

There are many drugs that contain the nitro group, but, although its presence may be a required feature of the drug

Table 2. Second order rate constants, k_2 , for the reaction of nitro radical anion of some nitro compounds of biological significance in different media obtained by electrochemical techniques

Compound	$k_2 \times 10^{-2}$ [Ms]$^{-1}$	Medium	Ref
Metronidazole	12 290 8400	43 % DMF pH 7.4 11% DMF pH 7.4 0 % DMF (extrapol.) pH 7.4	75,76
	66	50 % DMF pH 7.4	78
	110	Citrate buffer pH 7.4	79
	2.22	100% DMSO	80
PA 824	258.00	100% DMSO	80
Megazol	1.5	100 % DMF	81
	19	60 % DMF pH 9.0	82
Benznidazole	8.9	60 % DMF pH 9.0	82
Nifurtimox	0.53	60 % DMF pH 9.0	82
	4.86	100 % DMF	83
Nitrofurazone	112000	0 % DMF (extrapol.) pH 7.4	76
	12.00	60 % DMF pH 9.0	83
	7.5	100% DMF	83
NF 359	41.00	60 % DMF pH 9.0	83
	3.05	100% DMF	
Nifuroxime	6600	0 % DMF (extrapol.) pH 7.4	76
Nitrofurantoin	1.11	100 % DMF	84
Nifuroxazide	23.4	60 % DMF pH 10.5	85
Nitrendipine	960	0 % DMF (extrapol.) pH 7.4	86
	1.85	100 % DMF	90
	2.94	65 % DMF pH 9.0	91
	5.91	100 % DMF	91
Nimodipine	35	40 % DMF pH 8.2	87
	2.99	65 % DMF pH 9.0	91
	1.85	100 % DMF	91
	5.91	100 % DMF	90
Nicardipine	1.74	65 % DMF pH 9.0	88
	1.21	100 % DMF	90
	2.84	65 % DMF pH 9.0	91
Nifedipine	11	50 % DMF pH 7.4	89
	2.65	65 % DMF pH 9.0	91
	8.03	100 % DMF	90
Furnidipine	7.12	100 % DMF	90
	2.83	65 % DMF pH 9.0	91
	8.20	100 % DMF	91
Nisoldipine	2.85	65 % DMF pH 9.0	91
	7.12	100 % DMF	91
Loprazolam	4.55	65 % DMF pH 9.0	92
	1.20	100 % DMF	
Flunitrazepam	15.7	65 % DMF pH 9.0	92
Nimesulide	2.40	60 % DMF pH 9.0	93
Chloramphenicol	3750	0 % DMF (extrapol.) pH 7.4	76

Table 2. Second order rate constants, k_2 , for the reaction of nitro radical anion of some nitro compounds of biological significance in different media obtained by electrochemical techniques. (Continue)

Compound	$k_2 \times 10^{-2}$ [Ms]$^{-1}$	Medium	Ref
Flutamide	65.51	60 % DMF pH 7.4	94
	26.25	60 % DMF pH 8.0	
	25.60	60 % DMF pH 9.0	
	11.56	60 % DMF pH 10.0	
	5.64	60 % DMF pH 10.0	
	3.22	60 % DMF pH 11.0	
oxamniquine	63.51	0 % DMF pH 11	95

structure, it may not be directly involved in the mechanism of action. However, the aromatic nitro group may be connected to the incidence of toxic side effects associated with these compounds. This is the case of the nitroaromatic substituted 1,4-dihydropyridine derivatives (1,4-DHP) wherein the generation of free radicals could trigger toxic side effects. The first study in this scope was devoted to the $RNO_2^{\cdot-}$ generated from nitrendipine [86]. In this study the cyclic voltammetric technique allowed the determination of the second order rate constant and the half-life time for the $RNO_2^{\cdot-}$ generated in a mixed aqueous/DMF medium and by extrapolation in aqueous medium. Voltammetric studies of nimodipine using a mixed aqueous dimethylformamide (DMF) solvent have allowed to generate $RNO_2^{\cdot-}$. The cyclic voltammetry technique has been employed to study the tendency of $RNO_2^{\cdot-}$ to undergo further chemical reactions. This subsequent chemical reaction corresponds to a second-order process, a disproportionation reaction which is initiated electrochemically. Data for rate constants and half-lives at pH 8.2 were determined [87]. The cyclic voltammetric behavior of nicardipine was directed to the one-electron $RNO_2/RNO_2^{\cdot-}$ couple in mixed medium [88]. Analysis of this response as a function of scan rate yields information on the stability of the nitro radical anion. A second-order rate constant $k_2 = 174\ M^{-1}s^{-1}$ for the decomposition of the nitro radical anion from nicardipine and a half-life of 1.15 s were obtained. A similar electrochemical approach to the nitro radical anion formation from nifedipine was also applied [89]. Comparison of $RNO_2^{\cdot-}$ generated from several 1,4-DHP derivatives in aprotic medium shows that derivatives having the nitro group in the ortho position in the ring give less stable radicals when comparing with meta substituted [90]. Furthermore the nitro radical anion generation and stability of a series of both o-nitro and m-nitro substituted 1,4-DHP in mixed and aprotic media was studied [91]. The results show that in mixed medium the stability of both type of compounds was similar but in aprotic medium the $RNO_2^{\cdot-}$ from the o-nitro substituted derivatives was considerable less stable than the corresponding m-nitro substituted. The electrochemical study of the $RNO_2^{\cdot-}$ stability and the quantification of the second order kinetic constants for several other miscellaneous nitro compounds of biological significance as loprazolam [92], flunitrazepam [92], nimesulide [93], chloramphenicol [76], flutamide [94] and oxamniquine [95] has been also reported.

Recently, the use of the scanning electrochemical microscope (SECM) was proposed as a new method for measuring the kinetics of following chemical reactions in electrode processes. Particular attention to the application of the steady-state and chronoamperometric feedback modes was given to describe different redox mechanistic case. Bard and col. [96-100] had studied different mechanisms (E_rC_i, E_rC_{2i}, ECE and DISP 1), developing the current SECM theories in order to calculate heterogeneous and homogeneous constants. In all the cases a good agreement between the calculated homogeneous chemical constant, kc using SECM and other techniques were obtained. The use of the SECM eliminated many typical sources of experimental errors, e.g., the effects of the resistive potential drop in solution, the charging current and it enabled analytical measurements to be performed in the interfacial region [101]. On the other hand because SECM measurements can be made under steady-state conditions, short-time measurements are not needed, and contributions by possible adsorbed electroactive species are avoided, providing a great advantage in the study of free radicals of biological significance. The steady-state TG/SC mode, in particular, appears to be attractive for measuring fast chemical reactions, rate constants of the order of $4\times10^8\ M^{-1}s^{-1}$ which is in excess of those achievable via cyclic voltammetry, where higher rate constant than $1 \times 10^5\ M^{-1}s^{-1}$ are not possible to be studied.

In this way, the use of the SECM in TG/SC (tip generation/substrate collection) mode Fig. (**8A**) was proposed as a new alternative for measuring the kinetics of the chemical coupled reactions of the nitro radical anions generated from two different nitro compounds, PA-824 (a new antituberculosis agent) and metronidazole, MTZ, (a well known bactericidal agent) in DMSO. [102]. Ferrocene is generally used to determine the tip-substrate separation length, d, and the tip is scanned to the reduction of each nitro compound and then to the oxidation of ferrocene. The substrate electrode is held at a potential where the intermediate nitro radical anion, $RNO_2^{\cdot-}$, is oxidized (or collected) and the Fe^{3+}, from oxidized ferrocene, is reduced to Fe^{2+}. Thus the i_T is a measure of the nitro group reduction (or nitro radical anion generation) and the substrate current, i_S, is a measure of the amount of $RNO_2^{\cdot-}$ that reaches this substrate electrode before it reacts.

For a stable tip-generated species such as ferrocene, $i_S = -i_T$ or the collection efficiency $|i_S/i_T| = 1$. However, for a reactive tip-generated intermediate, i_S/i_T will depend upon the spacing between the tip and the substrate, d, then a plot of i_S/i_T against d/a (where "a" is the UME radius) can be employed to determine the rate constant for coupled chemical reactions of the $RNO_2^{\cdot-}$ intermediate. Thus,

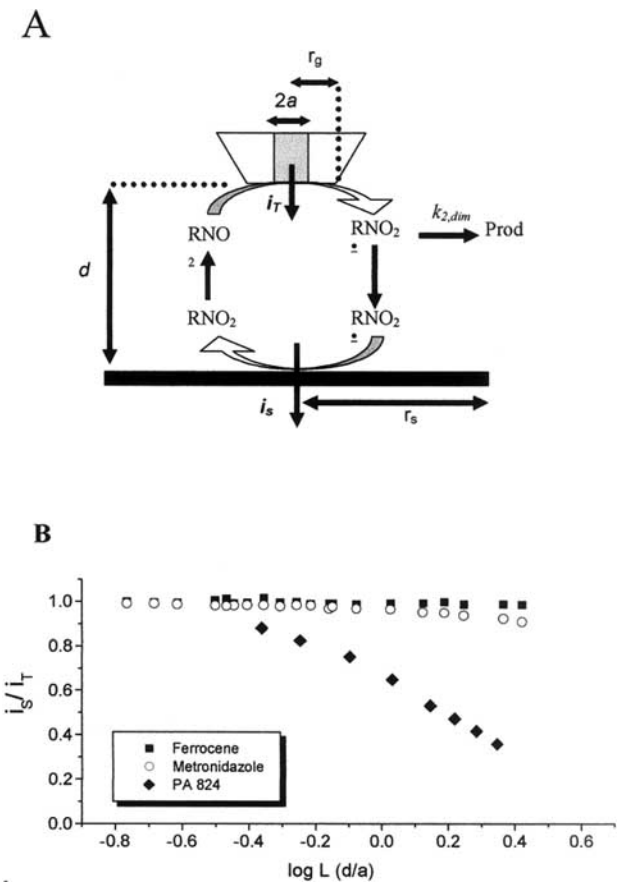


Fig. (8). A: SECM schematic diagram illustrating TG/SC mode for reductive dimerization of nitro radical anion; B: SECM collection efficiencies, i_S/i_T, versus the logarithm of the normalized distance, log L (d/a), nitrocompounds and ferrocene obtained in TG/SC mode at different tip-substrate separation,d. (Data from ref. 102 with permission of The Electrochemical Society, Inc.).

determining the collection efficiency, i_S/i_T, as a function of the gap space Fig. (**8B**), and comparing between three compounds, it is clear that MTZ nitro radical anion is a very stable species and that the substrate electrode is able to collect almost the 100% of the radical formed by the tip. On the other hand, the collection efficiency for PA-824 nitro radical anion is lower than 100 % at all the studied distances. Moreover, when the distance between the electrodes equals to the tip radius, the substrate is able to collect only the 60% of the PA 824 nitro radical anion produced in the tip. Using the SECM theory for dimerization process, a $k_{2,dim}$ values of $2.15 \pm 0.73 \times 10^4$ $[M \times s]^{-1}$ and 332 ± 74 $[M \times s]^{-1}$ for PA-824 and MTZ, were obtained.

For comparative purposes constant values using the cyclic voltammetry methodology of $k_{2,dim}$ values of $2.58 \pm 0.61 \times 10^4$ $[M \times s]^{-1}$ and 222 ± 38 $[M \times s]^{-1}$ for PA-824 and MTZ, were obtained. The agreement between the results from the well proved CV methodology and the recently developed SECM methodology support the validity of this new approach. Thus, the SECM technique results to be a modern new alternative for the study of the nitro radical anion electrogenerated from nitrocompounds of biological significance

REACTIVITY OF THE NITRO RADICAL ANION

Electrochemical techniques are able to detect and quantify the interaction between the $RNO_2^{\cdot}$ and its target (e.g. endo or xenobiotics of biological significance) as interaction will result in modifications to the current-voltage response. Addition of a target compound with which the $RNO_2^{\cdot}$ interacts will induce changes to the cyclic voltammetric response, measured as shifts in redox potential, changes to peak height and, in particular as alterations to the lifetime of the $RNO_2^{\cdot}$. One of the main advantages of the cyclic voltammetry in the study of the $RNO_2^{\cdot}$ is that this radical can be generated and its reactivity studied *in-situ*. To investigate the interaction of the $RNO_2^{\cdot}$ with any target, the rate constants for the decay reaction of the $RNO_2^{\cdot}$ must be determined both in the presence and in the absence of the target by following the $i_{p,a}/i_{p,c}$ value as described earlier [88]. The interactions between $RNO_2^{\cdot}$ formed from several nitro compounds of biological significance and targets as nucleic acid bases (thymine, adenine, cytosine, guanine, uracil) and aminothiols (glutathione, cysteamine, N-acetylcisteine, penicillamine, captopril) were studied. Some of these studies were only from qualitative character but several of them informed the calculation of the interaction constant. In table **3** we have summarized some of these results.

The evidence for the direct interaction of reduced metronidazole derivatives with DNA bases [103] and with aminothiols [104] using electrochemical techniques was reported. However no interaction rate constants were calculated for these interactions. However recently Mandal [79] investigated the interactions of the $RNO_2^{\cdot}$ from metronidazole with targets as thymine and cytosine using the cyclic voltammetric technique. Both the bases were found to react with the nitro radical anion with rate constants of 3.5×10^3 and 3.0×10^3 $M^{-1}s^{-1}$ for thymine and cytosine in aqueous buffer, respectively. In other recently published study the apparent rate constant of the interaction between $RNO_2^{\cdot}$ from furazolidone and cysteine in DMF + water mixtures was reported [105]. The interaction of glutathione, cysteamine, adenine and uracil as targets with a large series of 4-nitrosubstituted 1,4 DHP derivatives, capable to electrochemically generate the nitro radical anion, have been studied [91]. The interaction of the nitro radical anion of the 1,4-DHP derivatives with both thiols and nucleic acid bases includes hydrogen abstraction from the targets, originating a nitro derivative less reactive than the original nitro radical [106]. According to those results it is possible to conclude that the targets facilitate the disproportionation reaction of the nitro radical anion which regenerates the parent and generates the nitroso derivative, which undergoes a later reduction to produce the final hydroxylamine derivative as is shown in Scheme (**2**). On the other hand, the nitro radical anion from the nonsteroidal anti-inflammatory drug nimesulide interacts with the targets adenine, glutathione, uracil and cysteamine [93]. Furthermore, the reactivity of the $RNO_2^{\cdot}$ from the nonsteroidal antiandrogen flutamide towards thiol compounds and nucleic acid base were quantitatively

Table 3. Interaction rate constants, K_i , for the interaction of nitro radical anion from some nitro compounds of biological significance with several targets

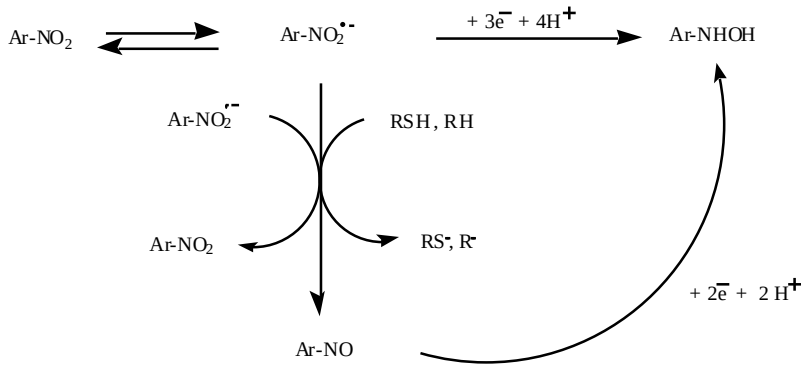
Compound	Target	Ki × 10^{-2}	Medium	Ref
Metronidazole	Thymine Adenine Cytosine Guanine	-	DMF-Water	103
Metronidazole	Cysteamine Glutathione	-	DMF-Water	104
Metronidazole	Thymine Cytosine	35 30	Citrate buffer, pH 7.4	79
Furazolidone	Cysteine	0.4 0.5	40% DMF pH 8.5 40% DMF pH 9.0	105
Nifedipine	Glutathione	171.1	65 % DMF pH 9.0	91
Nisoldipine		164.6		
Furnidipine		114.9		
Nitrendipine		95.3		
Nimodipine		81.4		
Nicardipine		85.5		
Nifedipine	Cysteamine	266.7		
Nisoldipine		196.0		
Furnidipine		171.5		
Nitrendipine		272.8		
Nimodipine		72.9		
Nicardipine		116.9		
Nifedipine	Adenine	125.2		
Nisoldipine		95.2		
Furnidipine		11.8		
Nitrendipine		71.6		
Nimodipine		17.6		
Nicardipine		34.4		
Nifedipine	Uracil	146.9		
Nisoldipine		23.7		
Furnidipine		12.4		
Nitrendipine		21.0		
Nimodipine		34.6		
Nicardipine		24.1		
Nimesulide	Glutathione	23.2	60 % DMF pH 9.0	93
	Cysteamine	9.10		
	Adenine	29.1		
	Uracil	11.4		
Flutamide	Glutathione	96.00	60 % DMF pH 9.0	94
	Cysteamine	155.34		
	N-acetylcisteine	23.19		
	Adenine	53.40		
	Uracil	113.51		
	Thymine	22.00		

assessed through the calculation of the respective interaction rate constants. The following order of reactivity towards the targets was as follows: cysteamine > uracil > glutathione > adenine > N-acetylcysteine > thymine.

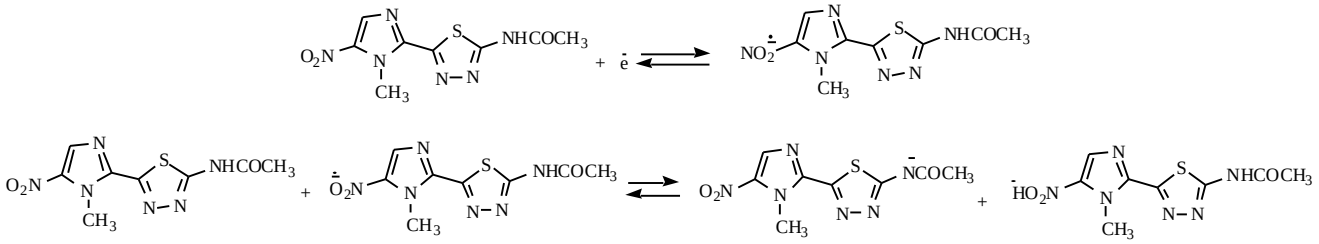
One of the most important *in-vivo* reactions of the $RNO_2^{\cdot-}$ is the reaction with oxygen, due to the well-known "futile" reduction or metabolism in aerobic conditions, with back-oxidation of the nitro radical anion [18]:

$$RNO_2^{\cdot-} + O_2 \rightleftharpoons RNO_2 + O_2^{\cdot-} \qquad (11)$$

The electrochemical techniques provides a very good tool for determining the equilibrium constant of the above



Scheme 2.



Scheme 3.

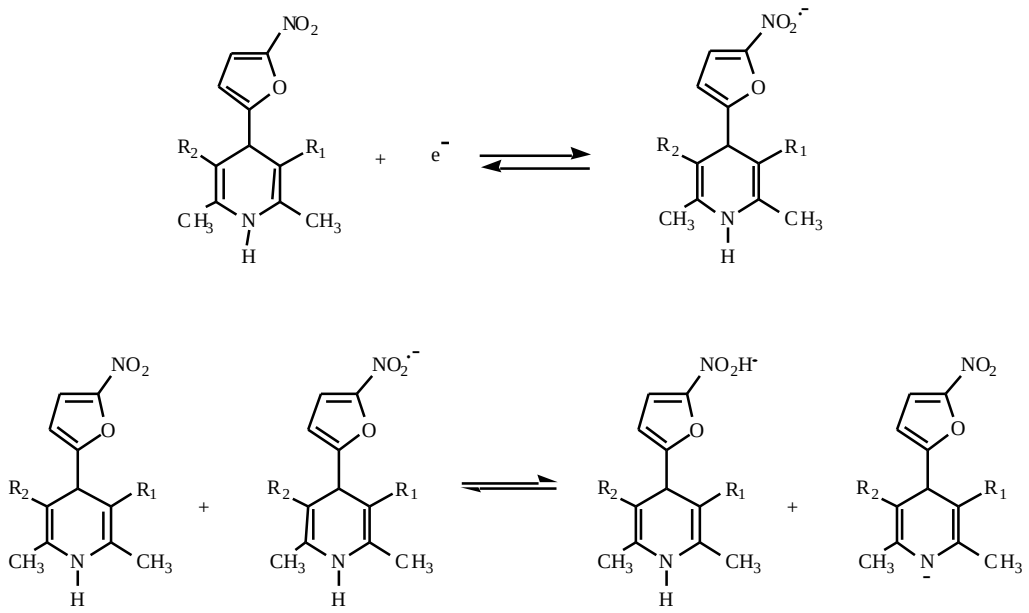
equation 11 (K_{11}). By substitution in the well-known thermodynamic relation $\Delta E = RT / nF \ln K$ the following equation, at 25ºC, is obtained [85]:

$$0{,}059 \log K_{11} = \{-0.155 - [E(RNO_2/ RNO_2^{\cdot-})]\} \qquad (12)$$

where -0.155 V is the redox potential for the couple $O_2/ O_2^{\cdot-}$ in the nonstandard state of 1 mM (equal as $RNO_2/ RNO_2^{\cdot-}$) in aqueous media, pH 7. Since $RNO_2^{\cdot-}$ is unstable in aqueous solution at pH 7, conventional electrochemical measurements such as polarographic half-wave potentials, $E_{1/2}$, cannot be equated with potentials for the one-electron couple, $E(RNO_2/ RNO_2^{\cdot-})$. However, electrochemical measurements of the cathodic peak potential, E_{PC}, using cyclic voltammetry in aprotic solvents [107] generally parallel the thermodynamic reversible one-electron potentials in water at pH 7. Consequently, with the single measurement of the E_{pc} of the one-electron reduction of the nitro compound it is possible to calculate the equilibrium constants for electron-electron transfer to oxygen for biologically important nitro compounds. The calculation of these equilibrium constants for some 5-nitrofurans derivatives was early reported [85]. From these results is possible to appreciate an agreement between the electrochemically calculated equilibrium constants and the *in-vivo* results of oxygen consumption giving validity to the determination.

Another important type of reactions that generates the $RNO_2^{\cdot-}$ is related with the basic character of this radical which is better observed in non-aqueous media. In fact this radical appears to be sufficiently basic in aprotic medium to react with hydrogen atoms with weakly acid character present in the same molecule thus originating an intermolecular acid-base reaction. Generally this phenomenon produces a change in the normal aspect of the cyclic voltammogram of the $RNO_2/ RNO_2^{\cdot-}$ couple with the existence of pre-peaks. In a recent study [108] two 5-nitroimidazolic compounds have been synthesized in order to study how the introduction of an acidic proton in the structure affected the electrochemical behavior of the nitro radical anion produced in aprotic media. One of these compounds, megazol Fig. (**2**), was compared with GC-361 which is totally similar to megazol but instead of the NH_2 substituent it has an acetamide substituent. Consequently, the main difference between both compounds is that, in the case of GC-361, the hydrogen neighbour to the carbonyl had an acidic character. In that paper it was proved that the acid proton in the acetamide group triggered an intermolecular reaction of the father-son type between the nitro radical anion generated from GC-361 (son compound) and the proper GC-361 (father compound) according to the scheme (**3**). In fact the nitro radical anion generated from the reduction of the GC-361 acts as a Brönsted base deprotonating the acetamide substituent generating the corresponding anion. The presence of two cathodic peaks is due to the anion (deprotonated compound) which is reduced at higher cathodic potentials than the neutral nitro compound.

Another similar type of reactions wherein the nitro radical anion acts as a Brönsted base was observed in the case of the nitroaryl substituted 1,4-DHP. The existence of father-son type reactions between nitrobenzene substituted 1,4-DHP was firstly reported in the nineties [109,90] but recently we have deepened in this matter with the aim to obtain an stable nitranion species from the parent 1,4-DHP derivatives [110]. Thus, we have synthesized new 1,4-DHP derivatives containing a nitrofuryl moiety as substituent at 4-position . In this case, the weakly acid character of the dihydropyridine and the sparseness of protons in aprotic media favored an intermolecular acid-base reaction between



Scheme 4.

the nitro radical anion and the proton in the N-1 position of the dihydropyridine ring, thus producing the corresponding nitranion according to the father-son type intermolecular reaction showed in Scheme **4**. Consequently, the nitro radical anion on the nitrofuryl moiety would act as a base deprotonating the N1 of the dihydropyridine ring. This finding is a nice manner to illustrate, in the same molecule, the acid-base concepts according to both Brönsted and Lewis theories. In fact the described mechanism implies the electrogeneration of a Lewis base (rich in electrons) as the nitro radical anion that afterwards acts as a proper Brönsted base deprotonating the 1,4-DHP ring. Considering the previous studies and the above results obtained with this new synthesized nitrofuryl 1,4-DHP derivatives, is possible to extrapolate this type of reactivity to all type of compounds containing, in the same molecule, a group capable to be electroreducible to a radical anion and a mildly acid hydrogen.

CONCLUSIONS

The nitro compounds of biological significance are a special type of compounds, whose particular biological functions (pharmacological or toxicological) are possibly related to their redox properties, which alternatively are associated to the stability or reactivity of the related reduced species. The nitro reduction products are essentially the radical anion, the nitroso and the hydroxylamine derivative which, depending on their reactivity in a particular reaction media, can be involved in a wide variety of chemical reactions. The predictive knowledge of this reactivity is important, because it opens the possibility to direct selectively of the electron transfer steps toward a specific situation, where chemical products or stable intermediates are formed.

From the different cases here analyzed, it is clear that the redox route for the nitro compounds reduction is strongly dependent of the reaction media. In aqueous media and depending on the pH, the tendency of the nitro compound will be the reduction via 4-electron generating an electrochemical signal. Electrochemical approaches permit to obtain high current / concentration ratios and low reduction potentials. These advantages have permitted the development of a great quantity of electroanalytical methods applied to nitro compounds of biological significance. In this review we have only described the survey of this topic in the last decade.

In a mixed and aprotic media is possible to direct selectively the reduction toward a stable nitro radical anion between the time schedule of the cyclic voltammetric technique. In this case the electrochemical techniques, particularly cyclic voltammetry and also recently scanning electrochemical microscopy, provide a very important tool to the quantitative determination of both generation and stability of the nitro radical anion. The generation of the $RNO_2^{\cdot-}$ depends fundamentally from the energy requirements to accept one electron by the nitro moiety and is reflected in his cyclic voltammetric half wave potentials, $E_{1/2}$. The stability of the $RNO_2^{\cdot-}$ depends fundamentally of the reaction media and is reflected in the value of the decay rate constants which can be quantitatively evaluated with the knowledge of the cyclic voltammetric theory for EC processes where the following chemical reaction corresponds to dimerization or disproportionation.

Also the electrochemical techniques were able to detect and quantify the interaction between the nitro radical anion derivative and several targets of biological significance. In fact, the addition of a target compound with which the nitro radial anion derivative interacts produced changes in the cyclic voltammetric response which had been used to quantitatively calculate the interaction constant value. Specifically the interaction of the nitro radical anion of the 1,4-DHP derivatives with both thiols and nucleic acid bases revealed hydrogen abstraction from the target, giving rise to a nitro derivative less reactive than the original nitro radical.

According to those results it is possible to conclude that the target facilitates the decay reaction of the nitro radical anion regenerating the parent compound and generating the nitroso derivative. The latter undergoes a subsequent reduction to produce the final hydroxylamine derivative. Consequently, one of the main advantages of the cyclic voltammetry in the study of the $RNO_2^{\cdot-}$ is that this radical can be generated and its reactivity studied *in-situ*.

Finally, it had been showed that the stability and reactivity of the nitro radical anion derivative is totally modified by direct interaction with oxygen or by the existence of intermolecular reactions of the father-son type between the nitro radical anion derivative and the parent nitro compound. In the first case with the single measurement of the E_{pc} of the one-electron reduction of the nitro compound it was possible to calculate the equilibrium constants for electron-electron transfer to oxygen for biologically important nitro compounds. In the second case the existence of an hydrogen atom with weakly acid character in some position of the nitro compound molecule and the sparseness of protons, in aprotic media, produced an intermolecular acid-base reaction between the nitro radical anion and the weakly acid proton in the nitro compound molecule, thus producing a father-son type reaction. This finding is a nice manner to illustrate, in the same molecule, the acid-base concepts according to both Brönsted and Lewis theories. In fact the described mechanism implies the electrogeneration of a Lewis base (rich in electrons) as the nitro radical anion that afterwards acts as a proper Brönsted base deprotonating the 1,4-DHP ring.

ACKNOWLEDGEMENTS

We are grateful to FONDECYT (Grant Nº 8000016 Líneas Complementarias) and Universidad de Chile for the financial support.

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