An electrochemical evidence of free radicals formation from flutamide and its reactivity with endo/xenobiotics of pharmacological relevance

Luis J. Núñez-Vergara*, Daniel Farias, S. Bollo, J.A. Squella

Laboratory of Bioelectrochemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, PO Box 233, Santiago, Chile

Abstract

This paper reports the feasibility of free radicals formation from flutamide by using cyclic voltammetry. The electrochemical characteristics and the reactivity of the one-electron reduction product from flutamide in mixed media with thiol compounds and the nuclei acid bases are characterized. Results from this paper show the thermodynamic feasibility of free radical formation expressed for both the cathodic peak potential and the second-order rate constant values.

The reactivity of the radical towards thiol compounds (glutathione, cysteamine, *N*-acetylcysteine) and the nuclei acid base, adenine, thymine and uracil were quantitatively assessed through the calculation of the respective interaction rate constants. Based on these results, the following tentative order of reactivity towards the xeno/endobiotics is as follows: cysteamine > uracil > glutathione > adenine > *N*-acetylcysteine > thymine.

The stability of the nitro radical anion electrochemically generated from flutamide showed a linear dependence with pH.

Keywords: Flutamide nitro radical anion; Cyclic voltammetry; Reactivity; Thiol compounds; Nuclei acid bases

1. Introduction

Flutamide (4-nitro-3-trifluoromethyl-isobutylanilide) is a non-steroid antiandrogen behaving as a competitive antagonist of the androgen receptor. The nitroaromatic compound is used in the treatment of advanced prostate cancer. However, the efficacy of this drug is somewhat overshadowed by the occurrence of hepatitis in a few patients [1]. Apparently, drug-induced hepatitis is often caused by the formation of reactive metabolites, which may lead to either toxic or immune hepatitis [2]. Unlike the related antiandrogen nilutamide, flutamide was not reduced into a nitroanion free radical by NADPH cytochrome *P*-450 reductase [3]. Instead, rat and human microsomal cytochrome *P*-450 oxidatively metabolized flutamide into electrophilic metabolite(s) [4].

The electrochemical reduction of nitrocompounds in aqueous media follows the general pattern involving a single four-electron step producing the hydroxylamine derivative [5–7]. On the other hand, the electrochemical reduction of such compounds is dramatically affected in mixed media. Thus, isolation of the one-electron reduction product, the nitro radical anion, is feasible [8,9]. Furthermore, under these experimental conditions, the reactivity of the nitro radical anion electrochemically generated from different nitroaromatic compounds using cyclic voltammetry and EPR could be assessed [10–14]. In aprotic media, these nitrocompounds are reduced to different products also involving the formation of free radical intermediates [15].

Toxicity of several nitroaromatic compounds has been related to the formation of reactive oxygen species and/or the formation of chemically reactive metabolites formed during the reductive metabolism of the nitro group. However, up to date, in the case of flutamide, the presence of free radicals due to its reductive biotransformation has not been found. Consequently, in the present paper, a study by cyclic voltammetry on formation and the characterization of the one-electron reduction product from flutamide in mixed media at different pH values is reported. In addition, its electrochemical characterization, the reactivity of the nitro radical towards different relevant pharmacologi-

E-mail address: lununez@abello.dic.uchile.cl (L.J. Núñez-Vergara).

cal targets, such as thiol compounds and nuclei acid-containing bases, is also informed.

2. Materials and methods

2.1. Drug and reagents

Flutamide (100% chromatographically pure) was provided by Schering Plough Laboratories (Santiago, Chile).

Dimethylformamide (DMF), spectroscopic grade, tetrabutylammonium iodide (TBAI), glutathione, cysteamine, thymine and adenine were purchased from Merck; *N*-acetylcysteine was obtained from Aldrich.

2.2. Cyclic voltammetry

Experiments were carried out in an INELECSA assembly PDC 1212, containing generator/potentiostat with an A/D converter interface attached to a 12-bit microprocessor and suitable software for totally automatic control of the experiments and data acquisition. A Gateway microcomputer was used for data control, acquisition, and treatment.

2.3. Electrodes

A BAS h.m.d.e. with a drop surface of 1.90 mm^2 was used as the working electrode and a platinum wire as a counter electrode. All potentials were measured against an Ag/AgCl electrode.

All cyclic voltammograms were carried out at a constant temperature of 25°C and the solutions were purged with pure nitrogen for 10 min before the voltammetric runs. The return-to-forward peak current ratio, I_{pa}/I_{pc}, for the reversible first electron transfer (the $Ar–NO_2/Ar–NO_2^{\cdot -}$ couple) was measured, varying the scan rate from 0.1 up to 10 V s^{-1}.

2.4. Methods

The experimental I_{pa}/I_{pc} ratios were calculated according to Nicholson's procedure, using individual cyclic voltammograms [16]. Furthermore, E_λ was selected to reduce the influence of the second cathodic peak. Fifteen runs with E_λ varying between -840 and -890 mV vs. Ag/AgCl did not show a significant variation on I_{pa}/I_{pc} values (coefficient of variation = 1.9%).

Kinetic reaction orders for the nitro radical anion was quantitatively assayed for first- and second-order coupled reactions according to a previously published study [17,18].

To ensure that changes in the voltammetric parameters (I_{pa}/I_{pc} ratio, E_{pc}, etc.) of flutamide by the addition of the nuclei acid bases, adenine, thymine, uracil and the thiol compounds were due to reaction between electrochemically generated intermediates, and not due to an electrode adsorption phenomenon, 150 μl of cyclohexanol was added to mixed media [19].

To quantitatively estimate the interaction rate constant (k_i) for the reaction between the nitro radical anion generated from flutamide and the endo/xenobiotics, we used a method previously developed in our laboratory [20].

2.5. Mixed media

From preliminary studies, to obtain the optimal mixed media, we have selected the following composition: 0.3 M aqueous borate buffer pH (7.4–12.0)/DMF: 40/60, 0.015 M aqueous trisodium citrate, 0.1 M TBAI and 0.3 M KCl. For the reactivity of radical with endo/xenobiotics, we used the same system as indicated below at pH 9.0.

2.6. pH in mixed media

pH measurements were corrected according to the following expression [21]: $pH^* - B = \log U_H^0$, where pH^* equals $-\log a_H$ in the mixed solvent, B is the pH meter reading. and the term $\log U_H^0$ is the correction factor for the glass electrode, which was calculated at different mixtures of DMF and aqueous solvent, according to a previously reported procedure [21].

2.7. Endo / xenobiotic solutions

Stock solutions of 10 mM of flutamide in DMF was prepared and protected from daylight to avoid photodecomposition. A routine of drug concentration of 5.0 mM for all the experiments was used. 50 mM stock solutions of all the tested compounds in borate buffer pH 9.0 were prepared to obtain solutions with concentrations ranging from 0.1 to 5 mM.

3. Results and discussion

The main goal of the present paper was both the electrochemical characterization of the nitro radical anion from flutamide and its reactivity with endo/xenobiotics of pharmacological relevance.

Some electrochemical and chromatographic characteristics of flutamide and their applications to the quantitative determination of the drug in tablets has been previously reported [22]. In that paper, the reduction of flutamide in an ethanol/aqueous medium at different pH was studied and some cyclic voltammetric experiments in mixed media were also reported. However, the optimal conditions for both the formation and characterization of nitro radical anion species, i.e. the reversible couple corresponding to the one-electron reduction product, were not studied.

3.1. Electrochemical characterization of the nitro radical from flutamide

Cyclic voltammetric experiments have permitted us to study the stability of the nitro radical anion from flutamide, as reflected in the return-to-forward peak current ratio. The use of an aqueous/DMF electrolytic medium has enabled us to characterize the $Ar–NO_2/Ar–NO_2^{\cdot-}$ couple electrochemically generated from flutamide. For these studies, a methodology based on the change in the I_{pa}/I_{pc} ratio has been used. So, if the species formed during the reduction (i.e. the forward scan) is stable on the electrochemical time-scale, then the return and forward current responses will be equal, giving an I_{pa}/I_{pc} ratio of unity. On the other hand, as the current response is proportional to concentration, we have the possibility to detect if the species formed by reduction could be capable of reacting with another molecule or it could be unstable on the electrochemical time-scale. In either case, there will be a decrease in the concentration of the species available for reoxidation, giving an I_{pa}/I_{pc} ratio lower than unity. In order to obtain the best experimental conditions to isolate the nitro radical anion both, the chemical composition of the electrolytic media and the electrochemical parameters were varied. As a consequence of such studies, a mixed medium containing 0.3 M aqueous borate buffer pH (7.4–12.0)/DMF: 40/60, 0.015 M aqueous trisodium citrate, 0.1 M TBAI and 0.3 M KCl to characterize the radical at different pH values was selected.

In Fig. 1, typical cyclic voltammograms corresponding to the one-electron reduction of flutamide at pH 7.4 at different sweep rates are shown. Under this pH condition and at a sweep rate of 1 V s^{-1}, a well-resolved reversible couple with an $E_{pa} = -725$ mV and $E_{pc} = -785$ mV, which corresponds to the one-electron reduction of the nitro group to the nitro radical anion (Scheme 1, Eq. (1)). Confirming this statement is the fact that the separation between the peaks had a value of 60 mV.

Subsequent reduction via an irreversible three-electron addition occurred at more negative potentials ($E_{pc} = -1200$ mV), according to Scheme 1, Eq. (2).

The stability of the radical species was also studied by changing the electrochemical conditions, e.g. the scan rate, the switching potential. Results show that as the scan rate increased, the I_{pa}/I_{pc} increased towards unity, a typical behavior for an irreversible chemical reaction following a charge-transfer step, i.e. an *E*lectrochemical *C*hemical (*EC*) process [12]. To check the order of the following chemical reaction, the dependence between the I_{pa}/I_{pc} ratio with the concentration of flutamide was assessed. As predicted by Olmstead et al. [18] for a second-order reaction (Scheme 1, Eq. (3)), the I_{pa}/I_{pc} ratio decreases parallel with the increasing concentration of the electroactive species. Furthermore, the cathodic peak potential also depends on flutamide concentrations and sweep rates, with $dE_{pc}/d\log c$ and $dE_{pc}/d\log v$ values varying between 19 and 22.5 mV. These values are in agreement with the theoretical value of 19.5 mV for an EC_i process where the chemical step follows a second-order kinetic [17]. The fact that the current ratio does not reach a value of unity can be ascribed to the effect of regeneration of the starting electroactive species as a consequence of a disproportionation reaction.

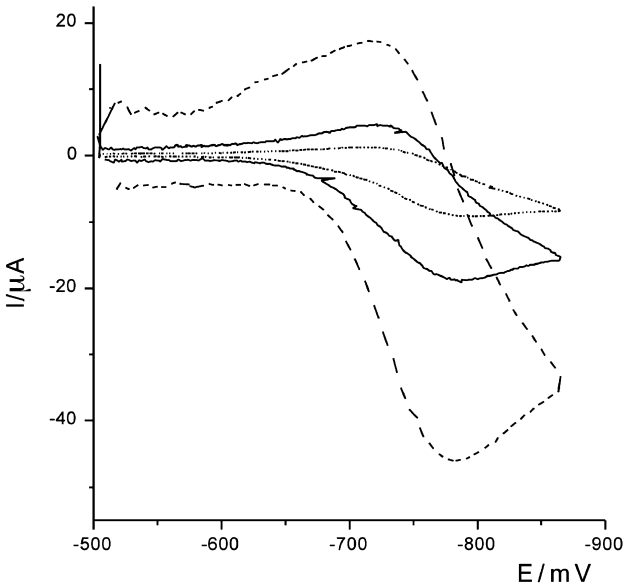
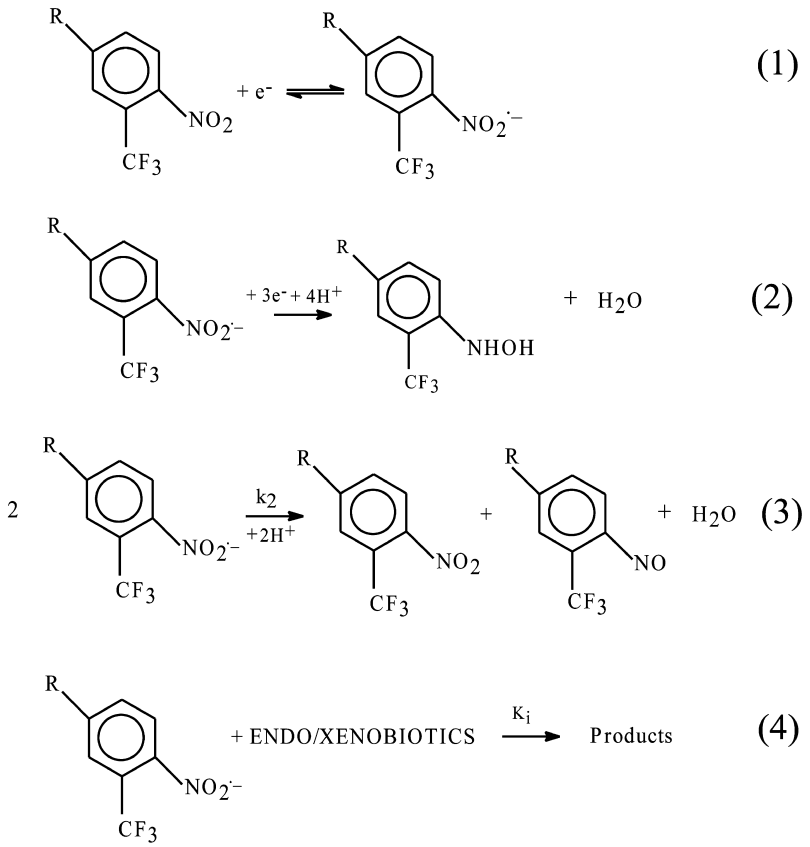


Fig. 1. Typical cyclic voltammograms corresponding to the nitro radical anion formation from flutamide at pH 7.4 at different sweep rates: (•••) 0.2 V s^{-1}, (–) 1.0 V s^{-1}, (- - -) 4.0 V s^{-1}.

The return-to-forward peak current ratio I_{pa}/I_{pc}, for the reversible first electron transfer (the $Ar–NO_2/Ar–NO_2^{\cdot-}$ couple) was measured for each cyclic voltammogram, varying the scan rate from 0.1 up to 10 V s^{-1} according to the procedure of Nicholson and Shain [16]. Using the theoretical approach of Olmstead et al. [18], the I_{pa}/I_{pc} values experimentally measured at each scan rate were inserted into a working curve for disproportionation to determine the ω parameter, which incorporates the rate constant, drug concentration and scan rate values. A plot of ω vs. τ resulted in a linear relationship described by:

$$\omega = k_2 C_0 \tau$$

where k_2 is the second-order rate constant for the decomposition of $Ar–NO_2^{\cdot-}$, C_0 is the flutamide concentration and τ is equal to $(E_\lambda - E_{1/2})/\nu$. 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 vs. τ. The assumption that the decomposition of $Ar–NO_2^{\cdot-}$ follows a second-order kinetics is supported by the linear relation between the kinetic parameter, ω, and the time constant, τ. In our case, confirming the second-order character of the following chemical reaction, plots of the kinetic parameter, ω, vs. the time constant, τ, at different pH were linear, with average correlation coefficients not lower than 0.980 (Fig. 2).



R= $(CH_3)_2$-CH-CONH-

Scheme 1. Equations involved in the mechanisms account for: (a) reduction of flutamide in mixed media (1,2), (b) natural decay of the nitro radical anion (3), (c) interaction between the nitro radical anion from flutamide and endo/xenobiotics (4).

The effect of pH on the I_{pa}/I_{pc} ratio and the anodic and cathodic peak potentials is shown in Table 1. Increasing the pH of the mixed media resulted in greater stability for the radical, as illustrated by an increase in the I_{pa}/I_{pc} ratio. The natural decay of $Ar–NO_2^{\cdot -}$ is by disproportionation reaction via the protonated radical $Ar–NO_2H^{\cdot}$, there-

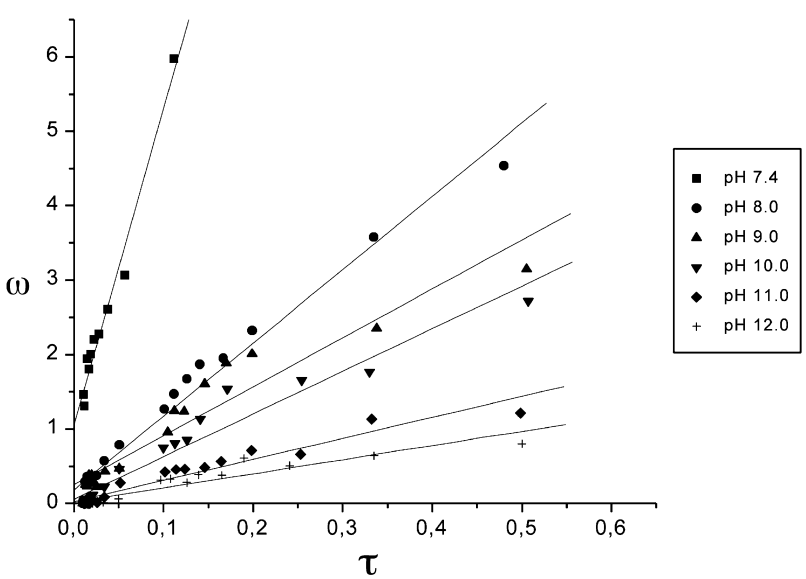


Fig. 2. Dependence of ω vs. τ at different pH values.

Table 1
Effect of pH on the I_{pa}/I_{pc} ratio for the flutamide $Ar–NO_2/Ar–NO_2^{\cdot-}$ couple and their corresponding peak potential values at a sweep rate of 1 V s^{-1}

	pH					
	7.4	8.0	9.0	10.0	11.0	12.0
I_{pa}/I_{pc}	0.739	0.876	0.893	0.908	0.956	0.963
$-E_{pa}$ (mV)	722	718	715	723	714	725
$-E_{pc}$ (mV)	788	786	783	789	781	788

fore, in alkali the un-protonated radical, $Ar–NO_2^{\cdot-}$ is more stable. This disproportionation reaction via the protonated radical, $Ar–NO_2H^{\cdot}$ has been previously documented by Kastening [23]. Also, no significant changes in potential peak values were observed. The pH-independence for cathodic peak potential values (E_{pc}) proves that no proton transfer precedes the electron transfer, i.e. both electractive species and products does not involved proton transfer. Consequently, the general sequence involved must be electron, proton, etc. [24]. To overcome the unreliability of pH measurements in mixed media, we have corrected our experimental values according to a previously reported method [21] as previously described in Methods section.

In Table 2, the second-order rate constants, k_2, and the corresponding lifetimes for the decay of the nitro radical as a function of pH are shown. Results from these studies demonstrated that decay constants decreased and consistently, the lifetime of the radical increased at more alkaline pH, indicating the stabilization of the radical species under this condition. Furthermore, as can be seen from Fig. 3, a plot of $\log k_2$ vs. pH gave a good linear dependence, with a correlation coefficient of 0.998.

3.2. Reactivity of the nitro radical anion from flutamide towards endo/xenobiotics of pharmacological relevance

Cyclic voltammetry becomes to be extremely useful, to detect and quantify the interaction between the one-electron reduction product from flutamide and relevant biological targets (endo/xenobiotics), which also resulted in modifications in the current–voltage response.

Table 2
Dependence of the second-order rate constants[a] (k_2) and half-lives[b] ($t_{1/2}$) corresponding to nitro radical anion from flutamide with pH

pH	k_2 $(M\ s)^{-1}$	$t_{1/2}$ (s)	r
7.4	6551	0.030	0.9487
8.0	2625	0.076	0.9879
9.0	2560	0.078	0.9919
10.0	1156	0.173	0.9949
11.0	564	0.354	0.9658
12.0	322	0.620	0.9802

[a] Second-order decay rate constant.
[b] Calculated values for a 5-mM concentration of flutamide.

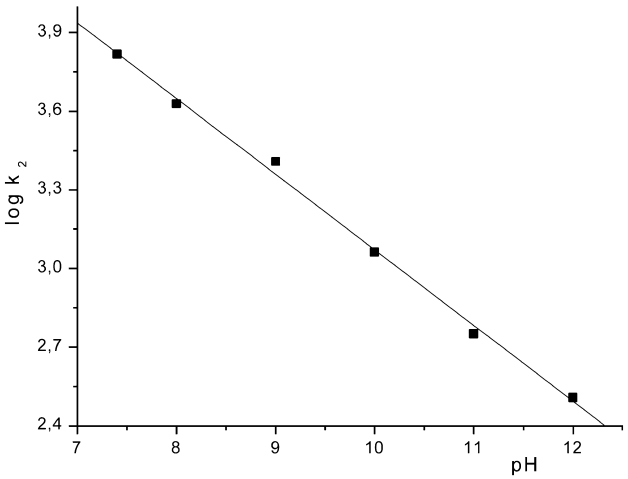


Fig. 3. Dependence of second-order rate constant (k_2) with pH.

The optimal medium should provide a good stabilization and separation of the nitro radical anion from other redox intermediates thus permitting us to study its reactivity with relevant biological targets in isolation. The mixed media that fulfills with the above characteristics was 0.3 M borate buffer/DMF (40/60), 0.015 M aqueous citrate 0.3 M KCL and 0.1 M TBAI at pH 9.0. To test the reactivity of the nitro radical anion from flutamide with the proposed endobiotics (glutathione, adenine, thymine and uracil) and the xenobiotics (*N*-acetylcysteine, cysteamine), a six-step method was applied. First, cyclic voltammograms of each endo/xenobiotic were carried out in both the same voltage and sweep rates in which the nitro radical appears. This study showed no peaks with any of the compounds assayed. Second, the comparison of different parameters of the cyclic voltammograms (i.e. I_{pc}, I_{pa}, E_{pc}, E_{pa}) both in the presence or in the absence of cyclohexanol did not show significantly differences in these electrochemical parameters for any of the endo/xenobiotics tested [19]. Therefore, we can conclude that under the experimental conditions here determined adsorption phenomena were negligible. Third, the characteristic linear dependence between ω and τ [18] for a second-order chemical reaction of the radical were obtained both in the absence (k_2) or in the presence of the endo/xenobiotics studied (k_i). However, concomitantly with the increase in the concentration of endo/xenobiotics, an increase in the slopes of the plots were also observed, always keeping correlation coefficients greater than 0.98. This last effect could be explained by the contribution of two different simultaneous competitive decay pathways, i.e. the natural decay of the radical (Scheme 1, Eq. (1)) and its reaction with the endo/xenobiotics (Scheme 1, Eq. (4)). Fourth, the effect of increasing concentrations of the studied compounds on the I_{pa}/I_{pc} ratios of the radical at different sweep rates was assessed. Fifth, from the slopes of the plots of ω vs. τ in the presence of the endo/xenobiotics, the k_{app} values were calculated. Finally, from plots $k_{app}/2k_2$ vs.

endo/xenobiotic concentrations and applying a previously described procedure [20], the interaction rate constants (k_i) were obtained.

To carry out a systematic study about the reactivity of the nitro radical anion with the different endo/xenobiotics, we have selected the mixed media at pH 9.0, taking into account the stabilization of the radical and the above-described results.

Effect of additions of endo/xenobiotics on the I_{pa}/I_{pc} ratios demonstrated that parallel with an increase of the concentration of such derivatives, a decrease in the current ratio at the different sweep rates was observed (Fig. 4). From these results, cysteamine becomes to be the most potent derivative with a decrease in the anodic peak current (I_{pa}) of 29% and the weakest effect corresponds to thymine (4.0%), at flutamide: endo/xenobiotic ratio of 1:1. Addition of cysteamine to flutamide solution for obtaining a flutamide:cysteamine 1:1 ratio, decreased the I_{pa}/I_{pc} value in 19% as compared with the control (without cysteamine).

On the other hand, at a similar concentration ratio (1:1), the nuclei-bases, uracil and adenine produced a significant decrease in the anodic peak current of 25.7% and 21.4%, respectively. The interaction rate constants (calculated according to the procedure described in Ref. [20] and their relationships for the reaction between the nitroanion from flutamide and the endo/xenobiotics at pH 9 are shown in Table 3. From this Table, it can be concluded that cysteamine shows the highest interaction rate constant (k_i) as compared with all the other tested derivatives. Based on these results at pH 9, the following tentative order of reactivity can be established: cysteamine > uracil > glutathione > adenine > *N*-acetylcysteine > thymine.

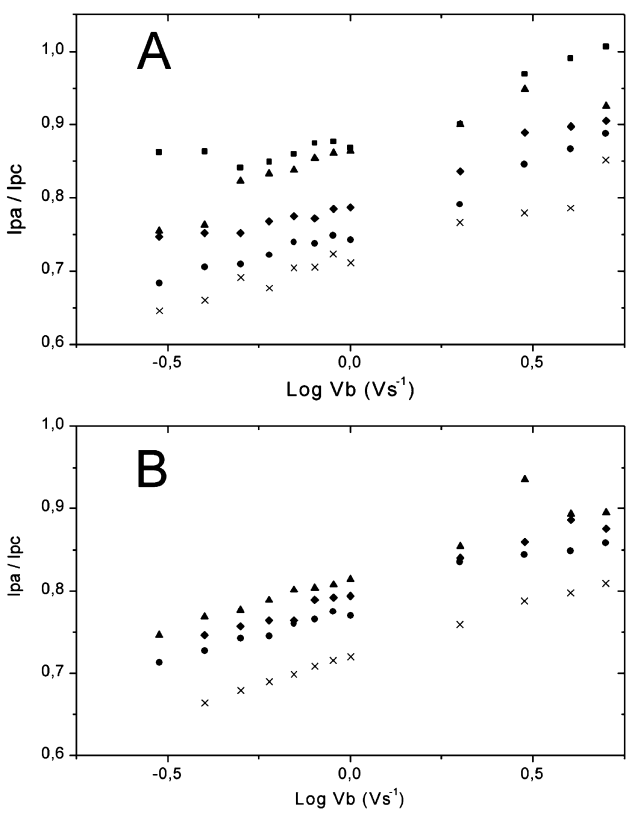


Fig. 4. Dependence of I_{pa}/I_{pc} ratio of the one-electron reduction product from flutamide with the logarithm of sweep rate in the absence and in the presence of: (A) glutathione, (B) uracil, (■) no addition, (▲) 0.2 mM, (♦) 1 mM, (●) 2 mM, (×) 5 mM.

Table 3
Interaction rate constants (k_i) for the reactivity between nitro radical anion from flutamide electrochemically generated with endo/xenobiotics of pharmacological relevance at pH 9.0 and a sweep rate of 1 V s^{-1}

Endo/xenobiotic	k_i $(M\ s)^{-1}$	k_i/k_2^a	$k_{iGSH}/k^b_{endo/xeno}$
Glutathione	9600	3.75	1.0
Cysteamine	15,534	6.07	0.6
N-acetylcysteine	2319	0.90	4.1
Adenine	5340	2.08	1.8
Uracil	11,351	4.43	0.9
Thymine	2200	0.86	4.4

[a] Second-order decay constant (k_2) = 2560 at pH 9.0.
[b] Constant ratio between the interaction rate constant for GSH and the interaction rate constants for the other tested endo/xenobiotics.

4. Discussion

In this work, the thermodynamic feasibility of free radical formation from flutamide expressed for the cathodic peak potential values is demonstrated.

Furthermore, a quantitative characterization of both the natural decay of the nitroanion from flutamide at different pH, even at physiological pH and its reactivity at pH 9.0 with relevant endo/xenobiotics was studied by using cyclic voltammetry.

The electrochemical generation of the radical species was achieved in a mixed medium, which resembles more adequately a physiological environment. The free radical species from flutamide in our anaerobic environment decays with a second-order kinetic at the different tested pH, with a constant value of 6551 $(M\ s)^{-1}$ at pH 7.4.

Taking into account our own results in which is demonstrated that the decay of the nitro anion radical from flutamide is pH-dependent and the findings of previous studies [12,25], it seems reasonable to propose for the decay of $Ar–NO_2^{\cdot -}$ the following reaction:

$$2Ar–NO_2H^{\cdot} \rightarrow Ar–NO_2 + Ar–NO + H_2O.$$

Studies on the reactivity of the nitro radical with biological targets were conducted in a mixed media at pH 9.0 because of the reliability of the current measurements. Results of these type of experiments show that with the exception of adenine and thymine, the interaction rate constants (k_i) for the resting endo/xenobiotics were higher than the respective rate constant of the natural decay of the radical (k_2) at pH 9.0, indicating that the species markedly

reacted with thiol compounds like GSH and cysteamine (Table 3). This latter compound appears as the most reactive with an interaction rate constant of 15,534 $(M\ s)^{-1}$. In addition to the redox characteristic of the free radicals from flutamide, prototropic equilibria control both the natural lifetime and its reactivity with biological targets. The protective role of thiols against the cytotoxicity of free radicals derived from nitroaryl compounds can arise from the reaction of $Ar–NO_2^{\cdot -}$ with $R–SH/R–S^-$, presumably by the following reaction:

$$Ar–NO_2^{\cdot -} + R–S^- + 2H^+ \rightarrow Ar–NO + R–S^{\cdot} + H_2O.$$

Recently [26], we have proved by ESR spectroscopy that in anaerobic conditions the nitro radical anion from nitrofurantoin is scavenged by glutathione. Furthermore, to assess the type of the interaction between GSH and the nitro anion radical, spin-trapping studies using DMPO were conducted. After the addition of the spin trap, a typical ESR spectrum of the adduct $GS^{\cdot}$ and DMPO appeared. These results substantiate the view that at least partially, the mechanism of the reaction involves the $GS^{\cdot}$ species.

Concerning the nuclei acid-containing bases, uracil was the most reactive endobiotic with a k_i value of 11,351 $(M\ s)^{-1}$. A possibility to explain our results could be that bases might act as proton donor, forming $Ar–NO_2H^{\cdot}$, leading to subsequent disproportionation according to the following:

$$Ar–NO_2^{\cdot -} + 2H^+ \ (\text{from bases}) \rightarrow 2Ar–NO_2H^{\cdot}$$
$$\rightarrow Ar–NO_2 + Ar–NO + H_2O.$$

Undoubtedly, this mechanism could be the most relevant in aprotic media, in which there is deficiency of H^+ ions, than that of aqueous or mixed media.

In conclusion, our results provide an electrochemical basis that thermodynamically supports the participation of the nitro group in the flutamide molecule and its one-electron reduction product in toxic events exerted by the drug.

Acknowledgements

This work was partially supported by Grant from FONDECYT (Project 8000016). The support from D.I.D from University of Chile is also acknowledged.

References

[1] L.J. Gomez, A. Dupont, L. Cusan, M. Tremblay, R. Suburu, M. Lemay, F. Labrie, Incidence of liver toxicity associated with the use of flutamide in prostate cancer patients, Am. J. Med. 92 (1992) 465–470.

[2] D.K. Wysowski, J.P. Freiman, J.B. Tourtelot, M.L. Horton, Fatal and nonfatal hepatotoxicity associated with flutamide, Ann. Intern. Med. 118 (1993) 860–864.

[3] D. Fau, D. Eugene, A. Berson, P. Letteron, B. Fromenty, C. Fisch, D. Pessayre, Toxicity of the Antiandrogen flutamide in isolated rat hepatocytes, J. Pharmacol. Exp. Ther. 269 (1994) 954–962.

[4] A. Berson, C. Wolf, C. Chachaty, C. Fisch, D. Fau, D. Eugene, J. Loeper, J.Ch. Gauthier, P. Beaune, D. Pompon, P. Maurel, D. Pessayre, Metabolic activation of the nitroaromatic antiandogen flutamide by rat and human cytochromes *P*-450, including forms belonging to the 3A and 1A subfamilies, J. Pharmacol. Exp. Ther. 265 (1993) 366–372.

[5] L. Baumane, J. Stradins, R. Gavars, G. Duburs, Mechanism and products of electrochemical reduction of 4-(nitrophenyl)substituted 1,4-dihydropyridines, Electrochim. Acta 37 (1992) 2599–2610.

[6] J.Y. David, J.P. Hurvois, A. Tallec, L. Toupet, Electrochemical behaviour of an unsymetrical 4-(*o*-nitrophenyl)1,4-dihydropyridine in protic media, Tetrahedron 51 (1995) 3181–3196.

[7] A. El Jammal, J.C. Vire, O. Nieto Palmeiro, G.J. Patriarche, Cyclic voltammetric study of some calcium antagonist dihydropyridines in aqueous media, Electroanalysis 4 (1992) 57–64.

[8] L.J. Núñez-Vergara, S. Bollo, C. Olea-Azar, P.A. Navarrete-Encina, J.A. Squella, Cyclic voltammetric and EPR spectroscopic studies of benzodiazepine: loprazolam and flunitrazepam, J. Electroanal. Chem. 436 (1997) 227.

[9] L.J. Núñez-Vergara, J.E. Guíñez-Castro, S. Bollo, J.A. Squella, Reactivity of the one-electron reduction product from nimodipine, nitrendipine and nicardipine with relevant biological thiols, Bol. Soc. Chil. Quim. 41 (1996) 363–370.

[10] J.A. Squella, P. González, S. Bollo, L.J. Núñez-Vergara, Electrochemical generation and interaction study of the nitro radical anion from nimesulide, Pharm. Res. 16 (1999) 161–164.

[11] A. Alvarez-Lueje, L. Naranjo, L.J. Núñez-Vergara, J.A. Squella, Electrochemical study of nisoldipine: analytical application in pharmaceutical forms and photodegradation, J. Pharm. Biomed. Anal. 16 (1998) 853–862.

[12] L.J. Núñez-Vergara, M.E. Ortiz, S. Bollo, J.A. Squella, Electrochemical generation and reactivity of free radical redox intermediates from *ortho*- and *meta*-nitro substituted 1,4-dihydropyridines, Chem.-Biol. Interact. 106 (1997) 1–14.

[13] L.J. Núñez-Vergara, S. Bollo-Dragnic, C. Olea-Azar, P. Santander, S. Gunckel, J.A. Squella, Reactivity of the nitro radical anion formation from nisoldipine with *N*-acetylcysteine: EPR spectroscopic and electrochemical evidences, Bol. Soc. Chil. Quim. 43 (1998) 231–239.

[14] L.J. Núñez-Vergara, G. Díaz-Araya, C. Olea-Azar, A.M. Atria, S. Bollo-Dragnic, J.A. Squella, Scavenging of the one-electron reduction product from nisoldipine with relevant thiols: electrochemical and EPR spectroscopic evidences, Pharm. Res. 15 (1998) 1690–1695.

[15] J.A. Squella, M. Huerta, S. Bollo, H. Pessoa, L.J. Núñez-Vergara, Electroreduction of 4-(nitrophenyl)substituted 1,4-dihydropyridines on the mercury electrode in aprotic medium, Electrochim. Acta 42 (1997) 2305–2312.

[16] R.S. Nicholson, I. Shain, A simple procedure to calculate the current ratio, Anal. Chem. 36 (1964) 1406.

[17] G. Bontempelli, F. Magno, G. Mozzochin, R. Seeger, Linear sweep and cyclic voltammetry, Ann. Chim. 79 (1989) 138–147.

[18] M. Olmstead, R. Hamilton, R.S. Nicholson, Theory of cyclic voltammetry for the disproportionation reaction and spherical diffusion, Anal. Chem. 41 (1969) 862–864.

[19] P.J. Declerck, C.J. De Ranter, Polarographic evidence for the interaction of reduced nitroimidazole derivatives with DNA bases, J. Chem. Soc., Faraday Trans. 1 83 (1987) 257–265.

[20] L.J. Núñez-Vergara, F. García, M.M. Domínguez, J. De la Fuente, J.A. Squella, In situ reactivity of the electrochemically generated nitro radical anion from nitrendipine with glutathione, adenine, uracil, J. Electroanal. Chem. 381 (1995) 215–219.

[21] A.G. Asuero, M.A. Herrador, A.G. González, Estimation of pH and autoprotolysis constants in mixtures of aliphatic amides with water: medium effect on the 4-aminoazobenzene system, Talanta 40 (1993) 479–484.

[22] A. Alvarez-Lueje, C. Peña, L.J. Núñez-Vergara, J.A. Squella, Electrochemical study of flutamide, an anticancer drug, and its polarographic, UV spectrophotometric and HPLC determination in tablets, Electroanalysis 10 (1998) 1043–1051.
[23] B. Kastening, Zur theorie der polarographischen strome bei dismutation des elecktrodenreaktionsprodukts, J. Electroanal. Chem. 24 (1970) 417–426.
[24] P. Zuman, The Elucidation of Organic Electrode Process, Academic Press, New York, 1969, pp. 1–21.
[25] A. Filali-Mouhim, D. Jore, P. Hickel, C. Ferradini, Radiolytic reduction of nifurtimox: the anaerobic mechanism, Radiat. Phys. Chem. 47 (1996) 125–130.
[26] L.J. Nuñez-Vergara, J.C. Sturm, C. Olea-Azar, P. Navarrete-Encina, S. Bollo, J.A. Squella, Free Radical Res. 32 (2000) 399–409.