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ELECTROCHEMICAL STUDY, ON MERCURY, OF A *META*-NITROARYLAMINE DERIVATIVE OF NOR-β-LAPACHONE, AN ANTITUMOR AND TRYPANOCIDAL COMPOUND[#]

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The electrochemistry of 2,2-dimethyl-(3*H*)-3-(*N*-3'-nitrophenylamino)naphtho[1,2-*b*]furan-4,5-dione ([Q]-$PhNO_2$), on mercury was investigated. The first peak is consistent with a quasi-reversible one-electron reduction of the *ortho*-quinone, forming [$Q^{\bullet -}$]-$PhNO_2$, while the second one, bielectronic, corresponds to the simultaneous reduction of the latter radical to a dianion and the nitro group to a nitro radical anion. The second order rate constant, k_{disp}, for the decay of [$Q^{\bullet -}$]-$PhNO_2$ is 15.188 x $10^3 \pm 827$ mol^{-1} L s^{-1} and the $t_{1/2}$ equals 0.06 s. $E^{1}{}_{7}{}^{Ic}$ values for [Q]-$PhNO_2$ and its precursor, nor-β-lapachone, are similar. The ease of semiquinone generation and its stability are parameters statistically relevant in the correlation biochemical/theoretical aspects.

Keywords: nitroquinones; semiquinone stability; kinetic constants.

INTRODUCTION

Quinones have been the subject of much interest due to their various biological activities,[1] mainly as antitumor compounds.[2] There are also several examples of quinone activity in Chagas' disease, caused by *Trypanosoma cruzi*.[3]

Two major mechanisms of quinone cytotoxicity have been proposed: stimulation of oxidative stress and alkylation of cellular nucleophiles, which encompass a large range of biomolecules,[1,4] such as DNA[1] and some enzymes, like topoisomerase[5] and protein tyrosine phosphatases.[6]

There are similarities among quinones and nitroheterocycles, since they catalyze the electronic transfer in biological processes, mainly through reduction reactions to generate radical anions (semiquinones and nitroaromatic radical anions), which depending on their stability, can transfer their free electrons to acceptor molecules. These acceptor molecules may be other electrogenerated species or molecular oxygen, which is, then, reduced to superoxide radical anion, generating conditions of oxidative stress inside the cell.

The knowledge and study of the electrochemical properties of the radical species generated in biological cycles of oxidation and reduction are essential to the biological activity understanding of this type of compounds. The cyclic voltammetric technique provides a very adequate procedure to evaluate the stability of the radical anions. Several nitro compounds of biological significance are capable to electrochemically generate nitro radical anion species and their stabilities have been studied using the cyclic voltammetric approach.[7] Summarizing, the radical anion 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 general Equations 1 and 2:

$$AB + e^- \leftrightarrows AB^{\bullet -} \tag{1}$$

$$m\,AB^{\bullet -} \rightarrow \text{Products} \tag{2}$$

wherein the chemical coupled reaction could be of first (m=1) or higher (m≠1) order depending on the reducing species and on the nature of the medium. The stability of the radical anion can be obtained from the evaluation of the kinetic constant of the chemical step. Olmstead et al. described a theory of cyclic voltammetry for both electrochemically initiated dimerization or disproportionation reactions.[8,9]

In this work we studied the electrochemical behavior of 2,2-dimethyl-(3*H*)-3-(*N*-3'-nitrophenylamino)naphtho[1,2-*b*]furan-4,5-dione, ([Q]-$PhNO_2$) (Figure 1) and its precursor nor-β-lapachone (2,2-dimethyl-(3*H*)-naphtho[1,2-*b*]furan-4,5-dione, Q). The compounds Q and [Q]-$PhNO_2$[10,11] are cytotoxic against several cancer lines.[10,11] The trypanocidal activity of [Q]-$PhNO_2$ was also reported and it is higher than benznidazole (IC_{50}/24 h (μM) 86.3 ± 4.6 vs. 103.6 ± 0.6 of benznidazole).[12]

In a previous paper, the electrochemical behavior of [Q]-$PhNO_2$ in acetonitrile solution on a glassy carbon electrode was studied.[13] Therein, it was shown that both quinone and nitro functions are reduced independently as *quasi*-reversible one-electron transfer processes and afford different radical ion species. Depending on the reduction potential, the presence of a radical anion or a biradical dianion was confirmed by performing *in-situ* Electrochemical-Electron Spin Resonance (E-ESR) experiments.[13]

In this paper, we carried out a comprehensive cyclic voltammetric study on a mercury electrode in order to study the reduction of [Q]-$PhNO_2$ and the consequent formation of radical anions. The focus of this work is the quantitative measurement of the stability

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[#]This work is dedicated to Prof. Hans Viertler, a leader and enthusiastic researcher on Organic Electrochemistry, on the occasion of his 70th birthday.

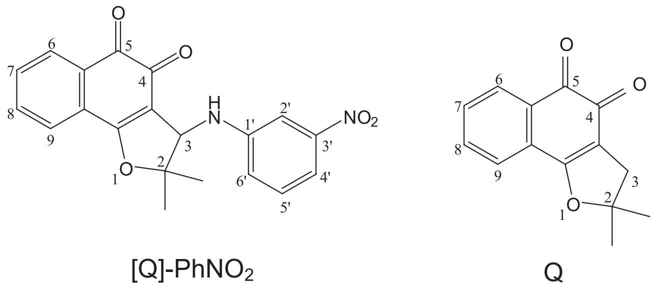

Figure 1. *[Q]-PhNO$_2$: 2,2-dimethyl-(3H)-3-(N-3'-nitrophenylamino) naphtho[1,2-b]furan-4,5-dione and [Q]: 2,2-dimethyl-(3H)-naphtho[1,2-b] furan-4,5-dione*

constants applying the Olmstead's methodology.[8,9] Apparently, the nitrogen substituent bonded to the quinone nucleus exerts influence on the redox properties of the quinone moiety improving the cytotoxic activity.[14] It was also demonstrated that to be a good trypanocidal compound, the molecule must be a good electron acceptor to reach easily the essential semiquinone state, the one really statistically relevant parameter in the correlation biochemical/theoretical aspects.[15]

EXPERIMENTAL

2,2-Dimethyl-(3*H*)-naphtho[1,2-*b*]furan-4,5-dione (nor-β-lapachone, Q)[10] and 2,2-dimethyl-(3*H*)-3-(*N*-3'-nitrophenylamino) naphtho[1,2-*b*]furan-4,5-dione ([Q]-PhNO$_2$) were synthesized according to a procedure earlier described.[11]

Solutions for cyclic voltammetry were prepared starting from a 0.02 mol L^{-1} stock solution of ([Q]-PhNO$_2$) in DMSO. Final solutions in the voltammetric cell were prepared by diluting an appropriate quantity of the stock solution in order to obtain a final concentration of 1 mmol L^{-1}. All the voltammetric experiments were obtained after bubbling with N$_2$ for 10 min in the cell before each run.

Experiments in aprotic medium were performed in DMSO with 0.1 mol L^{-1} tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

Apparatus and methods

Voltammetric curves were recorded on an electrochemical analyzer type BAS 100B/W (Bioanalytical System) attached to a PC computer with appropriate software (BAS 100W) for total control of the experiments and data acquisition and treatment. A static mercury drop electrode (SMDE) was used as the working electrode and platinum wire as the counter electrode. All potentials were measured against Ag/AgCl/NaCl (3 mol L^{-1}).

For the kinetic analysis carried out in aprotic medium, the return to forward peak current ratio I_{pa}/I_{pc} for the reversible electron transfer was measured for each cyclic voltammogram, varying the scan rate from 0.1 up to 2 V s^{-1}, according to the procedure described by Nicholson.[16] Using the theoretical approach of Olmstead and Nicholson,[8,9] 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 the rate constant, ([Q]-PhNO$_2$) concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by Equation 3:

$$\omega = k \cdot C^{o}\, \tau \qquad (3)$$

where *k* is the rate constant for the decomposition of the corresponding radical species, C° is the ([Q]-PhNO$_2$) concentration and $\tau = (E_{\lambda} - E_{1/2})/v$, wherein E_{λ} is the switching potential, $E_{1/2}$ is the half wave potential and *v* is the sweep rate. Consequently, we can obtain the rate constant for the dimerization or disproportionation of the ([Q]-PhNO$_2$) radical anion from the slope of the straight line ω *vs.* τ.

RESULTS AND DISCUSSION

2,2-Dimethyl-(3*H*)-3-(*N*-3'-nitrophenylamino)naphtho[1,2-*b*] furan-4,5-dione ([Q]-PhNO$_2$) was reduced in a non-aqueous medium containing 0.1 mmol L^{-1} TBAP in DMSO when it is subjected to a cyclic voltammetric (CV) experiment on Hg electrode. As observed in Figure 2, two cathodic peaks are displayed. The first peak has a cathodic peak potential, E_{pIc} of −0.570 V and the second one has an E_{pIIc} of −1.120 V, at a sweep rate of 0.5 V s^{-1}. These cathodic peaks displayed two corresponding backward peaks at potential of -0.495 and -1.020 V, respectively, showing an apparently reversible behavior. With the aim to study the voltammetric behavior at different time scales of the experiment, measurements were made at different sweep rates. From the voltammograms shown in Figure 3, the difference between cathodic and anodic peak potentials, Δ*E*p, for these apparently reversible couples exceeds the theoretical value of 0.060 V for a one-electron reversible process. With the obtained results, a totally reversible reaction for these peaks was discarded since, in fact, the involved electron transfer would obey a mechanism related to a coupled chemical reaction.

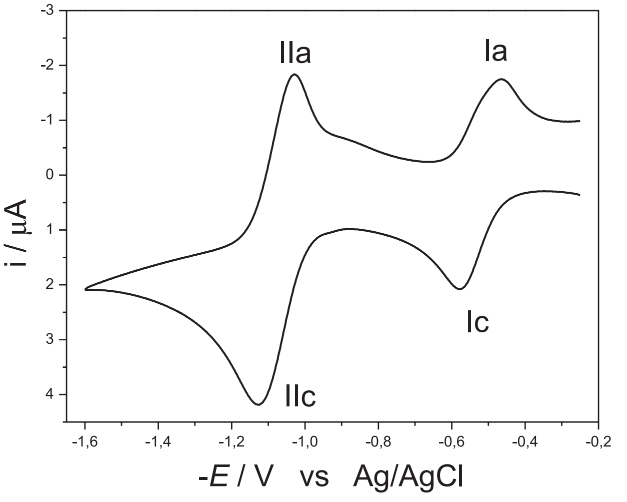

Figure 2. *Cyclic voltammogram (CV) of 1 mmol L^{-1} [Q]-PhNO$_2$, in DMSO with 0.1 mol L^{-1} tetrabutylammonium perchlorate (TBAP) vs. Ag/AgCl/NaCl (3 mol L^{-1}). Hg electrode. Sweep rate 0.5 V s^{-1}*

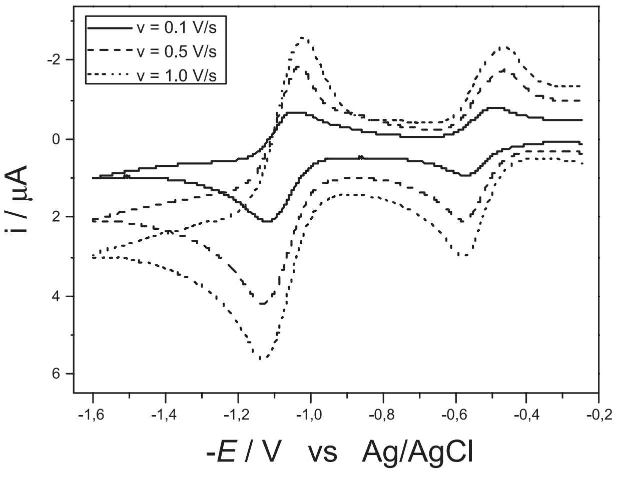

Figure 3. *CV of 1 mmol L^{-1} [Q]-PhNO$_2$ in DMSO with 0.1 mol L^{-1} TBAP vs. Ag/AgCl/NaCl (3 mol L^{-1}), at different sweep rates. Hg electrode*

The observed two peaks can be ascribed to the following: the first peak is consistent with a first one-electron reduction of the *ortho*-quinone function in order to form the semiquinone radical according to Equation 4:

$$[Q]\text{-}PhNO_2 + e^- \leftrightarrows [Q^{\bullet-}]\text{-}PhNO_2 \quad (4)$$

According to the previous paper for the reduction of the same compound in acetonitrile, on glassy carbon electrode,[13] the second cathodic peak appearing at a more negative potential would correspond to the further one-electron reduction of the electrogenerated radical anion in order to obtain a quinone-nitro biradical dianion, according to Equation 5:

$$[Q^{\bullet-}]\text{-}PhNO_2 + e^- \leftrightarrows [Q^{\bullet-}]\text{-}PhNO_2^{\bullet-} \quad (5)$$

however, in the present case, this behavior must be discarded because the second peak has a 2:1 height ratio, when compared with the first one-electron reduction peak, evidencing a two-electron reduction for the second peak. In order to support this hypothesis we have examined the voltammogram of nor-β-lapachone (Q) (Figure 1). The cyclic voltammogram of Q shows two peaks (Ic and IIc) at similar potentials of [Q]-PhNO$_2$, however it differs in the peak current of the second peak. In fact, the second peak on the voltammogram of Q shows approximately one half of the peak current of the equivalent peak on the voltammogram of [Q]-PhNO$_2$ (Figure 4). Considering that the height of the wave obtained by tast polarography can be directly correlated with the number of electrons causing the wave, we have repeated the same comparison but using tast polarography. From the polarograms on Figure 5, we can prove that the second peak of Q corresponds to a one-electron wave instead of the two-electron wave in the case of the [Q]-PhNO$_2$. Obviously the difference can be ascribed to the presence of the nitro group. Therefore the second peak of reduction of [Q]-PhNO$_2$ corresponds to the reduction of the semiquinone [Q$^{\bullet-}$]-PhNO$_2$ to a dianion quinone-nitro radical according to Equation 6, in which the reductions of the semiquinone and the group nitro occur at similar potential. The polarographic E$_{1/2}$ value of 3-nitroaniline [-1.21 V *vs.* SCE = -1.25 V vs. Ag/AgCl/NaCl (3 mol L^{-1})], obtained in acetonitrile, is herein included for comparison purposes, showing that the potential is adequate for the reduction of the nitroaromatic function.[17]

$$[Q^{\bullet-}]\text{-}PhNO_2 + 2\,e^- \leftrightarrows [Q^{=}]\text{-}PhNO_2^{\bullet-} \quad (6)$$

It is well known that the use of Hg instead of GCE as a working electrode in the CV experiments allows a considerable decrease in the capacitive current, thus increasing the working sweep rates and consequently enlarging the time scale of the experiment. In this scope, we can study the couple corresponding to the above Equation 4, in order to obtain information about the stability of the radical.

Cyclic voltammograms of the isolated first couple at different sweep rates are shown in the inset of Figure 6. From these data, we obtained a linear relation between the cathodic peak current and the logarithm of the sweep rate with slope of 0.40. This slope value is indicative of a diffusion-controlled process since it is near the theoretical value of 0.50 for this type of process.

From the dependence of the current ratio (I_{pa}/I_{pc}) with the sweep rate (Figure 6) we can deduce a mechanism with a coupled chemical reaction following the one-electron reduction, according to the following EC overall mechanism for the first couple (Equations 4 and 7):

$$[Q]\text{-}PhNO_2 + e^- \leftrightarrows [Q^{\bullet-}]\text{-}PhNO_2 \quad (4)$$

$$[Q^{\bullet-}]\text{-}PhNO_2 \rightarrow \text{Product} \quad (7)$$

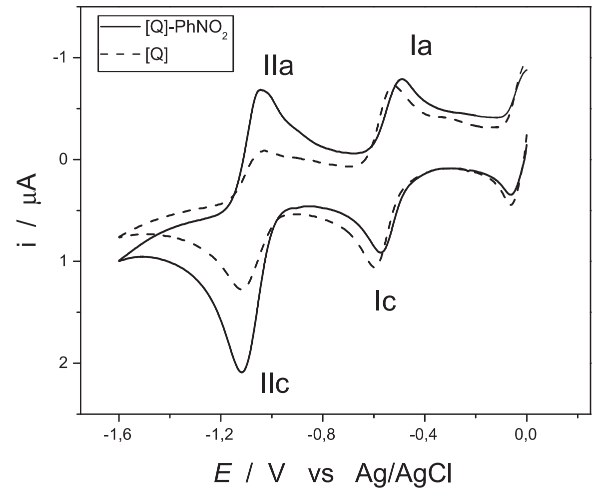

Figure 4. *CV of 1 mmol L^{-1} [Q]-PhNO$_2$ and Q in DMSO with 0.1 mol L^{-1} TBAP vs. Ag/AgCl/NaCl (3 mol L^{-1}). Hg electrode. Sweep rate 0.1 V s^{-1}. Obs.: At 0 V, the pair of peaks is from the supporting electrolyte*

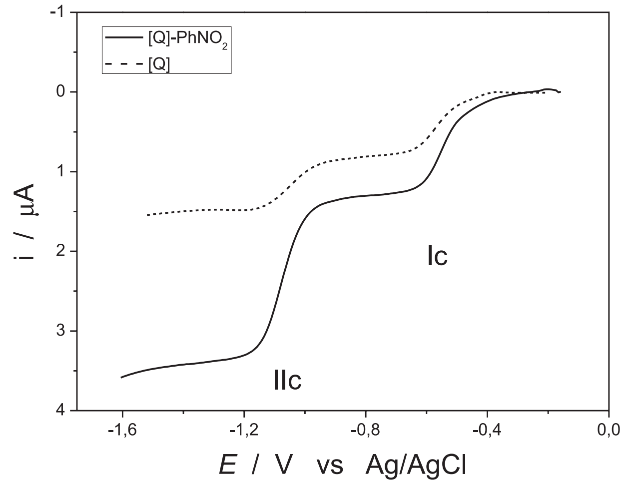

Figure 5. *TAST polarography of 1 mmol L^{-1} [Q]-PhNO$_2$, and Q in DMSO with 0.1 mol L^{-1} TBAP vs. Ag/AgCl/NaCl (3 mol L^{-1})*

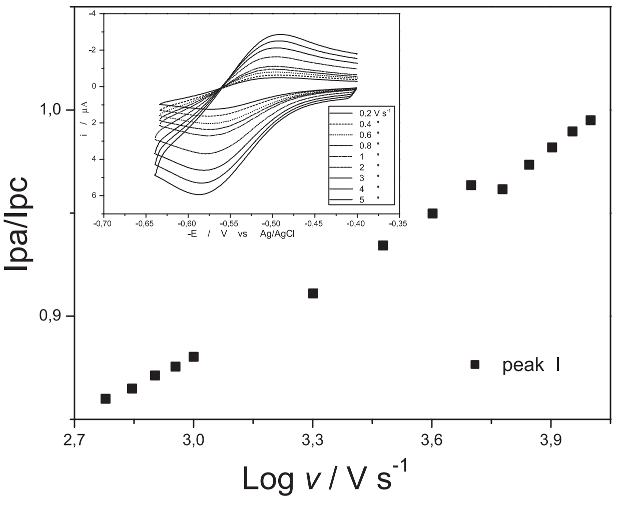

Figure 6. *Dependence of the current ratio on sweep rate for the first couple of the reduction of [Q]-PhNO$_2$. Inset: The corresponding cyclic voltammograms for the couple [Q]-PhNO$_2$ / [Q$^{\bullet-}$]-PhNO$_2$. CV obtained from solutions containing 1 mmol L^{-1} [Q]-PhNO$_2$ in DMSO with 0.1 mol L^{-1} TBAP, vs. Ag/AgCl/NaCl (3 mol L^{-1}), Hg electrode, at different sweep rates*

We have applied the theoretical approach of Olmstead *et al.*[8,9] for a disproportionation and dimerization coupled reactions. The procedure implies the experimental measurement of the I_{pa}/I_{pc} values at several scan rates for the first couple (Equations 1 and 4). The obtained I_{pa}/I_{pc} values were inserted into both working curves, for disproportionation[8] and for dimerization[9] in order to obtain the corresponding ω values and, then, the graphs ω *vs.* τ lines. From these graphs we found that the line obtained from the disproportionation working curve adjusted better, *i.e.*, there is a better correlation coefficient than for dimerization. Consequently we can conclude that the semiquinone radical species would decay by a disproportionation reaction. Furthermore, we have calculated the second order rate constant (k_2), for the species. Plots of the kinetic parameter, ω *vs.* the time constant τ, were linear (Figure 7). From the slope of this straight line we obtained the second order rate constant, k_{disp}, for the decay of [Q$^{\bullet-}$]-PhNO$_2$ species. We found a k_{disp} value of 15.188 x 10^3 ± 827 mol^{-1} L s^{-1}, $t_{1/2}$ = 0.06 s.

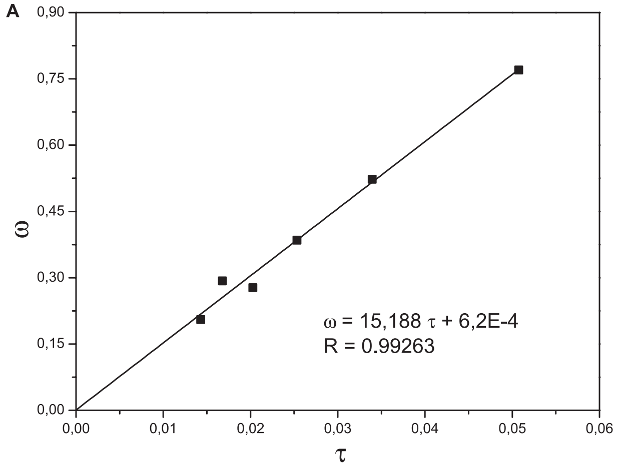

Figure 7. *Plot of the kinetic parameter, ω, with the time constant, τ. A) For the couple [Q]-PhNO$_2$ / [Q$^{\bullet-}$]-PhNO$_2$*

The above data in aprotic medium not only described the situation in that medium but also permit to obtain biological significant parameters as the E^1_7.[7,21] The E^1_7 value is a thermodynamic reversible potential for couples that accounts for the energy necessary to transfer one electron to an electroactive group to form a radical anion, at pH 7, in aqueous medium, and it is considered indicative of radical anion formation *in vivo*.[18] The correlation can be obtained from $E_{1/2}$ from different solvents and electrodes, once the system is reversible.

Furthermore, a correlation between the cathodic peaks potentials (E_{pc}), obtained in aprotic media, with the E^1_7 values obtained by pulse radiolysis, has been previously described for nitro radical anions.[19] On the other hand, since the quinone $E_{1/2}$ values correlate directly with E^1_7 values,[20] we have used the previously reported relation between E^1_7 and $E_{1/2}$ for some quinones (i.e. phenantroquinone, β-lapachone, 1,4-naphthoquinone and naphthazarin),[22] in order to obtain an extrapolated value for the first electron reduction to the semiquinone. Consequently, with the obtained experimental values of the E_{pIc} ($E_{1/2}$ = (E_{pIc} - E_{pIa}) / 2), in aprotic medium, we calculated the E^1_7 values for the reduction to [Q]-PhNO$_2$ and Q species from the first peak. The E^1_7 values obtained for the radical species are displayed in Table 1.

The obtained E^1_7 values for [Q$^{\bullet-}$]-PhNO$_2$ and Q are very similar meaning that both compounds have the same tendency for radical anion formation *in vivo*. Furthermore a similar tendency is showed for related quinones such as phenanthroquinone, 1,4-naphthoquinone and β-lapachone.[22] From the comparison of the results for [Q]-PhNO$_2$ and Q, apparently, the nitroaniline substituent bonded to the quinone nucleus, in a non-conjugated manner, did not exert influence on the energy necessary to transfer one electron to the quinone group to form the semiquinone, at pH 7, in aqueous medium.

Table 1. $E_{1/2Ic}$ and E_1^{7Ic} values for the first reduction peak (Ic) obtained from CV of 1 mmol L^{-1} [Q]-PhNO$_2$ and Q in DMSO, vs. Ag/AgCl/NaCl (3 mol L^{-1}), on Hg electrode and comparison with E^1_7 values from related quinones

Quinones	$-E_{1/2}^{Ic}$ (V)	$-E^{1\ Ic*}_7$ (V)
Q	0.560	0.140
[Q]-PhNO$_2$	0.538	0.138
Phenanthroquinone*	0.460	0.124
1,4-Naphthoquinone*	0.488	0.140
β-lapachone*	0.604	0.145

*Data obtained from refs. 21, 22

CONCLUSIONS

The knowledge and study of the electrochemical properties of the radical species generated in biological cycles of oxidation and reduction are essential to the biological activity understanding of drugs and xenobiotics chemically related to quinones, nitroaromatics, nitroheterocyclics and iminiums. They may provide information both on their electron affinity and the possibility of one-electron transfer *in vivo*, and thus on their possible toxicity due to the production of oxygenated free radicals.[23] The obtained $E^{1\ Ic}_7$ values for the first reduction wave of [Q]-PhNO$_2$ and Q are very similar, meaning that both compounds have the same tendency for radical anion formation *in vivo*. The ease of semiquinone generation and its stability are parameters statistically relevant in the correlation biochemical/theoretical aspects.

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