CYCLIC VOLTAMMETRY AND DIGITAL SIMULATION OF THE ELECTROREDUCTION OF *ORTHO*- AND *META*-NITRO ARYL 1,4-DIHYDROPYRIDINES

S. BOLLO, L.J. NUÑEZ-VERGARA AND J.A. SQUELLA*

Bioelectrochemistry Laboratory, University of Chile, P.O. Box 233, Santiago 1, Chile.
e-mail- asquella@ll.ciq.uchile.cl

SUMMARY

Cyclic voltammetry (CV) and digital simulation methods are utilized to elucidate the mechanism of electrochemical reduction of a series of nitro aryl 1,4-dihydropyridines in protic (aqueous) and mixed media. The experimental voltammograms show different behavior for the reduction of these compounds. In protic media, one cathodic and one anodic peak are observed, but in mixed media a new redox couple appears, indicating a change in the redox mechanism.

We have determined that the position of the nitro group (*ortho-*, *meta-*) in nitroaromatics directly affects both the electron transfer rate (heterogeneous rate constant, $k°_{10}$, $k°_{12}$) and the energetics of the electron transfer (E° values).

According to this study, the splitting observed in the cyclic voltammograms, when aprotic solvent or inhibitors are added to a nitroaromatic aqueous solution, is due to a) a change in the kinetics of the first electron transfer reaction $(k°_s)$ from an irreversible to a reversible process, b) to a change in the energetics of the electron transfer and c) to the presence of a dismutation chemical reaction

KEY WORDS: Nitroreduction, dihydropyridines, cyclic voltammetry, simulation.

RESUMEN

Voltametría cíclica y simulación computarizada fueron utilizadas para elucidar el mecanismo de la reducción electroquímica de una serie de nitro aril-1,4-dihidropiridinas en medio prótico (acuoso) y mixto. Los voltamogramas experimentales revelaron un comportamiento reductivo diferente de los compuestos en los dos medios. En el medio prótico, se observaron una señal catódica y otra anódica, en cambio, en medio mixto una nueva señal reversible, par redox, fue observada, indicando claramente un cambio en el mecanismo redox.

* To whom correspondence should be addressed.

Por otro lado, se ha determinado que la posición del grupo nitro (*orto-*, *meta-*) en el anillo aromático afecta directamente tanto la velocidad de la transferencia electrónica (constantes heterogéneas de velocidad, $k°_{10}$, $k°_{12}$) y la energética de la transferencia (valores de $E°$).

De acuerdo a este estudio, el desdoblamiento observado en los voltamogramas cíclicos, cuando el solvente aprótico o inhibidores son agregados a la solución acuosa del compuesto, es debida a: a) un cambio en la cinética de la primera transferencia electrónica ($k°_s$) desde un proceso irreversible a uno reversible, b) a un cambio en las energéticas de las transferencias electrónicas y c) a la presencia de la reacción química de dismutación del anión radical nitro.

PALABRAS CLAVES: Nitroreducción, dihidropiridinas, voltametría cíclica, simulación.

INTRODUCTION

Even though nitroaromatic compounds have been investigated since the beginnings of organic electrochemistry[1], it is still a very active area of research because there are some features of the reduction process which need further investigation. For example, there is a strong relationship between the redox pathway and the reaction media. In fact, the reduction of nitroaromatics in aqueous solution in the absence of inhibitors on mercury electrode involves a single four-electron step[2-8] according to:

$$ArNO_2 + 4e^- + 4H^+ \rightarrow ArNHOH + H_2O \qquad (1)$$

In acidic media the phenylhydroxylamine derivative is further reduced to the phenylamine derivative in a second two-electron step. In alkaline solutions or in the presence of inhibitors[4,7] and also in aprotic solvents[8-11], the electrode process described by eqn. (1) splits into two steps according to:

$$ArNO_2 + e^- \rightleftharpoons RNO_2^{\cdot-} \qquad (2)$$

$$ArNO_2^{\cdot-} + 3e^- + 4H^+ \rightarrow ArNHOH + H_2O \qquad (3)$$
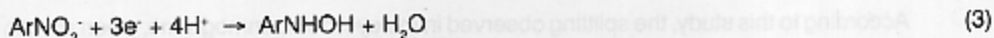
The cyclic voltammetric response corresponding to eqn. (1) is a cathodic irreversible peak and the responses corresponding to eqns. (2) and (3) are a reversible one-electron couple and an irreversible three-electron peak, at more negative potentials. Summarizing, the change in the media produces the splitting of the initial irreversible four-electron peak in two new signals. However, the exact nature of this splitting is a controversial matter. Some explanations to this point are as follows: Kalendyk *et al.*[7] ascribed this splitting to the rate of the very high (therefore, reversible) one electron reaction even in the presence of inhibitors, while the subsequent three-electron step are slow (therefore, irreversible). B. Kastening[3] assumed that in the presence of inhibitors, the second electron rate transfer decreases enough to produce a separate wave at more negative potentials, whereas the one-electron transfer remains reversible even in the presence of strong inhibitors. Zuman *et al.*[5] assumed that the presence of DMF decreases the rate of the surface protonation probably due to competitive adsorption. Finally, Tocher *et al.*[8] assumed that the change in the redox pathway is due to the following: in aqueous systems, the first one-electron transfer (E_1) is reversible, but occurs at more negative reduction potentials than the following three-electron transfer (E_2) to give the hydroxylamine derivative, i.e., $E_1 < E_2$. Thus, upon formation, the nitro radical anion immediately undergoes further reduction steps to the hydroxylamine derivative, and no intermediate reduction products are detected. The addition of DMF shifts E_2 to more

negative values, so that $E_1 > E_2$. An independent reduction process to form the nitro radical anion is then clearly evident. This one-electron transfer step is fast (reversible) and diffusion controlled and there is agreement in the literature about this point.

In recent years, several authors have used simulators for the cyclic voltammetric response to obtain or confirm mechanisms, to determine thermodynamic and kinetic parameters which are difficult or impossible to attain by other methods[12-16].

In this paper we have used cyclic voltammetry (CV) to obtain the experimental voltammograms for each reaction media of a series of nitro aryl 1,4-dihydropyridines (Figure 1) which are classified into two groups: *ortho* nitro derivatives (*o*-nitro) and *meta* nitro derivatives (*m*-nitro). Moreover, we have also used a CV simulator DIGISIM 2.1[012] in order to obtain the simulated cyclic voltammograms. Digital simulation of CV curves were also run in order to obtain the mechanism in the different media and estimate heterogeneous rate constants and formal electrode potential for individual electrochemical steps along with the rate constants for the homogeneous chemical steps.
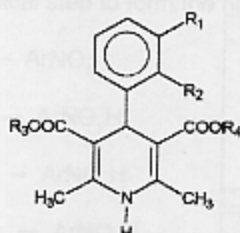

FIG. 1. Chemical structures of *ortho*- and *meta*-nitro aryl 1,4-dihydropyridines.

|	R_1	R_2	R_3	R_4
NITRENDIPINE	NO_2	H	CH_3	CH_3
NIMODIPINE	NO_2	H	C_2H_6	$(CH_2)_2OCH_3$
NICARDIPINE	NO_2	H	CH_3	$(CH_2)_2N(CH_3)(CH_2C_6H_5)$
NIFEDIPINE	H	NO_2	CH_3	CH_3
NISOLDIPINE	H	NO_2	CH_3	$CH_2CH(CH_3)$
FURNIDIPINE	H	NO_2	CH_3	$CH_2C_4H_7O_3$

EXPERIMENTAL

Materials: Analytical grade reagents were used without further purification. The nitroaromatic 1,4 dihydropyridine drugs, nifedipine, nisoldipine, furnidipine, nimodipine, nitrendipine and nicardipine were obtained from national pharmaceutical laboratories. CV measurements were performed in 1 mM of each drug in 40:60/Britton Robinson buffer (pH = 7.4):Ethanol (protic media solution) and 1 mM of each drug in 40:60/citrate buffer (pH = 9):DMF (mixed media solution). The ionic strength was kept constant at 0.3 M with KCl.

Apparatus: CV curves were recorded with a BAS CV-50W electrochemical system equipped with a Controlled Growth Mercury Electrode (CGME) and linked with a GATEWAY 2000 PC for acquisition and processing of data. A three electrode cell containing a hanging mercury drop electrode (HMDE) as a working electrode, a Silver/Silver Chloride electrode supplied with a bridge for mixed solvents as a reference electrode and a platinum wire as an auxiliary electrode. CV simulated curves were obtained using software DIGISIM 2.1* CV simulator for WINDOWS*. The software was run using a GATEWAY 2000 PC.

RESULTS AND DISCUSSION

1.1 The reduction of nitroaryl-1,4-dihydropyridines in protic media

CV curves taken using 1 mM of each of the nitrocompounds in protic media (40:60/Britton Robinson buffer (pH = 7.4):Ethanol) show a single irreversible, 4-electron reduction peak (A) about -700 mV for the *m*-nitro and -795 mV for the *o*-nitro (Figure 2a and 2b, respectively). In both cases
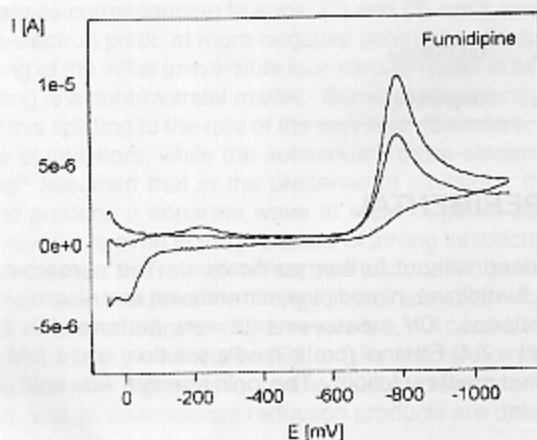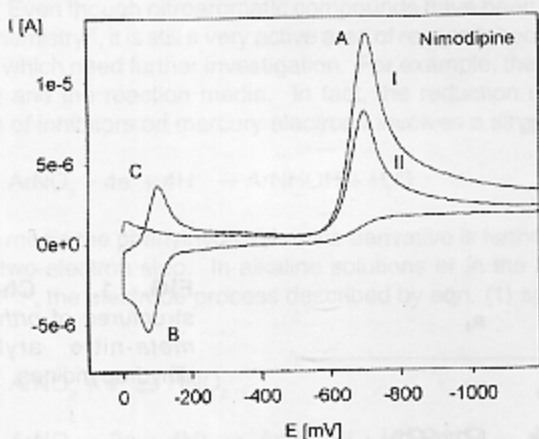

FIG. 2. Experimental CV curves obtained from 1 mM solution of Nimodipine and Furnidipine in protic media; 40:60/Britton Robinson buffer (pH = 7.4):Ethanol. Sweep rate 1 V/s.
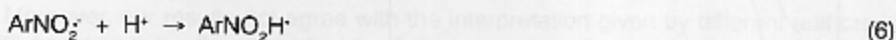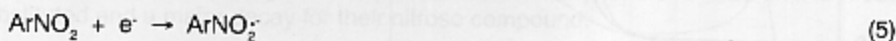
the hydroxylamine derivative is generated according to the equation:

$$Ar\text{-}NO_2 + 4e^- + 4H^+ \rightarrow Ar\text{-}NHOH + H_2O \tag{1}$$
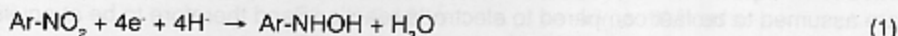
In the reverse sweep, anodic peaks (B) at 20 mV for the o-nitro and -65 mV for the m-nitro are observed (Figure 2). This corresponds to the oxidation of the hydroxylamine derivative, which is formed during the first negative-going sweep, to form nitroso compound in a process involving two electrons. A new reduction peak, C, appears in Figure 2, at about -220 mV for the o-nitro and -100 mV for the m-nitro, on the second negative going potential scan which is paired with the anodic peak B and is thought to be the reduction of newly formed nitroso compound:

$$Ar\text{-}NO + 2H_2O + 2e^- \rightleftharpoons Ar\text{-}NHOH + 2OH^- \tag{4}$$
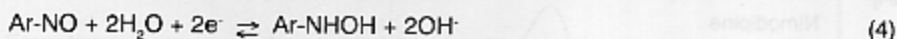
The overall electrode process involves four electrons and four protons. The reduction of nitrocompounds to hydroxylamine is modeled in this way by two electron transfer step (5) and (7) followed by an irreversible chemical step to form the nitroso compound (9):

$$ArNO_2 + e^- \rightarrow ArNO_2^- \tag{5}$$

$$ArNO_2^- + H^+ \rightarrow ArNO_2H^\cdot \tag{6}$$

$$ArNO_2H^\cdot + e^- \rightarrow ArNO_2H^- \tag{7}$$

$$ArNO_2H^- + H^+ \rightarrow ArNO_2H_2 \tag{8}$$

$$ArNO_2H_2 \rightarrow ArNO + H_2O \tag{9}$$

and later, two further electron transfers (10) and (12) to produce the hydroxylamine derivative:

$$ArNO + e^- \rightarrow ArNO^{\cdot-} \tag{10}$$

$$ArNO^{\cdot-} + H^+ \rightarrow ArNOH^\cdot \tag{11}$$

$$ArNOH^\cdot + e^- \rightarrow ArNOH^- \tag{12}$$

$$ArNOH^- + H^+ \rightarrow ArNHOH \tag{13}$$

Finally we include an irreversible condensation reaction (14) between the hydroxylamine and the nitroso derivatives, as previously reported in protic media[16]:

$$Ar\text{-}NO + Ar\text{-}NHOH \rightarrow Ar\text{-}N(O)=N\text{-}Ar + H_2O \tag{14}$$
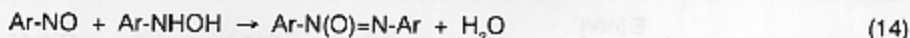
To stimulate the voltammograms in this media, we considered the results obtained in previous work for the reduction of other nitroaromatics such as nitrobenzene[17] and p-nitrobenzoic acid[16] in protic media. In this work the authors proved, by using time-resolved SERS technique, that the E° values of the first two electron transfers were substantially lower than the experimental E_p values; i.e., $E^\circ = -0.38$ V, $E_p = -0.8$ V. Furthermore, E_p values differentiated from E° values, which implied the existence of irreversible processes. Then, in our simulation, the first two E° values were chosen according to these results and the other two were determined directly from the experimental voltammograms assuming reversible electron transfer processes.

The CV curves were simulated for scans between 50 mV and -1100 mV. All diffusion coefficients were assumed to be equal to D = 1x10⁻⁵ cm²/s. Water is taken as the proton source, and all hydrolytic reactions are assumed to be fast compared to electrode reactions and therefore to be at equilibrium.

In Figure 3, the current curves indicate the "best fit" obtained from the experimental CVs (Figure 2) by means of the DigiSim® data-fitting routine. The following parameters were used in order to obtain these curves:
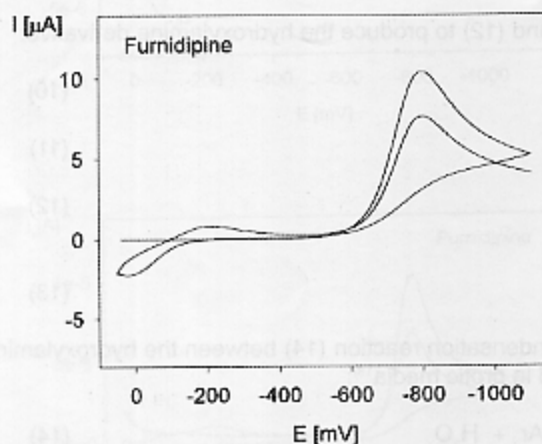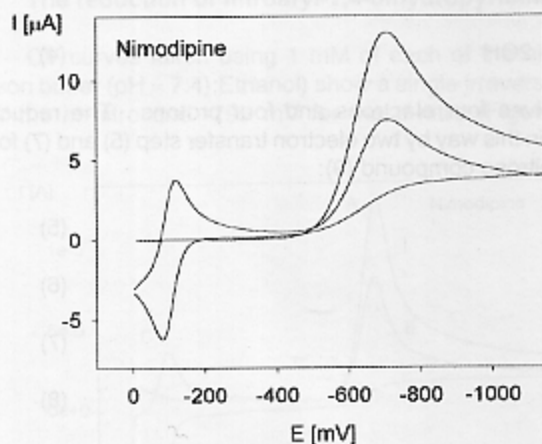
FIG. 3. Simulated CV curves obtained from 1 mM solution of Nimodipine and Furnidipine in protic media; 40:60/Britton Robinson buffer (pH = 7.4):Ethanol. Sweep rate 1 V/s.

Nimodipine:
(m-nitro)

$E^\circ_5 = -0.28$ V	$k^\circ_5 = 1.1 \times 10^{-5}$ cm s⁻¹
$E^\circ_7 = -0.20$ V	$k^\circ_7 = 2.9 \times 10^{-5}$ cm s⁻¹
$E^\circ_{10} = -0.10$ V	$k^\circ_{10} = 2.5 \times 10^{-2}$ cm s⁻¹
$E^\circ_{12} = -0.10$ V	$k^\circ_{12} = 1$ cm s⁻¹
	$k_{c9} = 10^2$ s⁻¹
	$k_{c14} = 2$ s⁻¹

| **Furnidipine:**	$E^\circ_5 = -0.38$ V	$k^\circ_5 = 2.4 \times 10^{-5}$ cm s^{-1}
(o-nitro)	$E^\circ_7 = -0.35$ V	$k^\circ_7 = 8.1 \times 10^{-5}$ cm s^{-1}
	$E^\circ_{10} = -0.12$ V	$k^\circ_{10} = 10^{-2}$ cm s^{-1}
	$E^\circ_{12} = -0.11$ V	$k^\circ_{12} = 2.5 \times 10^{-2}$ cm s^{-1}
		$k_{c9} = 3 \times 10^2$ s^{-1}
		$k_{c14} = 40$ s^{-1}

We have also tested the validity of the parameters set by showing that the variations in their values do have measurable effects on the simulated peak potential and peak currents (the obtained variation coefficient values were near to 2% for all tested parameters). Similar values were obtained for other four related nitroaromatics (two o-nitro and two m-nitro).

From these experimental and simulated results, we can conclude that, first, the energetics of the electron transfers (Ep and E° values) are greater in the o-nitro derivatives, an effect due to a steric hindrance of the nitro group with the 1,4-dihydropyridine moiety. Secondly, the main differences in the obtained values for k°_{12} and k_{c14} were significantly (in the case of k°_{12} there was a 400 fold difference in the o-nitro compounds), indicating a more irreversible tendency for the Ar-NO/Ar-NHOH couple in the ortho substituted and a major decay for their nitroso compound.

Moreover, our results not agree with the interpretation given by different authors in previous papers[3,6-8], because they all suggest that the first electron transfer in aqueous media is fast, i.e., reversible, and we have found values for the rate constants of around 10^{-5} cm/s (irreversible) for all derivatives. On the other hand, our values agree with data obtained by Shi et al.[16] for the electrochemical reduction of p-nitrobenzoic acid in protic media with rate constant of 1.08×0^{-5} cm/s for the first electron transfer.

1.2 The reduction of nitroaryl-1,4-dihydropyridines in mixed media

CV curves obtained with 1 mM of each nitrocompound in mixed media (40:60/Citrate buffer (pH = 9.0):DMF) shows the effect of adding DMF to a solution, with a splitting of the 4-electron peak obtained in protic media, i.e., the reduction of the hydroxylamine derivative now occurs via two clearly resolved stages for all derivatives (Figure 4). The first reduction step involved the reversible transfer to 1-electron to form the nitro radical anion, i.e., the ArNO$_2$/ArNO$_2^-$ couple, with a clear difference in reduction potential between o-nitro and m-nitro derivatives. The second step involved the subsequent reduction via an irreversible 3-electron addition occurring at more negative potentials, according to:

$$ArNO_2 + e^- \rightleftharpoons ArNO_2^- \qquad (2)$$

$$ArNO_2^- + 3e^- + 4H^+ \rightarrow ArNHOH + H_2O \qquad (3)$$
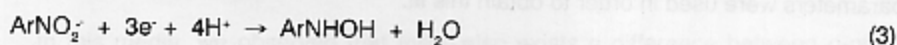
The CV curves were simulated considering a mechanism that involved reaction 5-13. We also considered the dismutation of the nitro radical anion (15) as a chemical step in the mechanism[18,19]:

$$2ArNO_2^- + 2H^+ \rightarrow ArNO_2 + Ar-NO + H_2O \qquad (15)$$
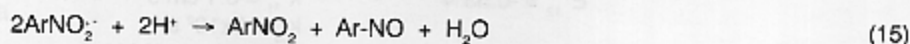
Moreover, in this case, we assumed that the first electron transfer is reversible, and therefore the E° value should be closer to the Ep.
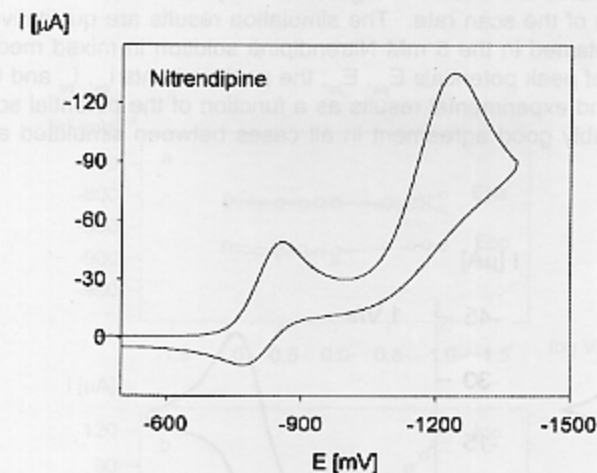
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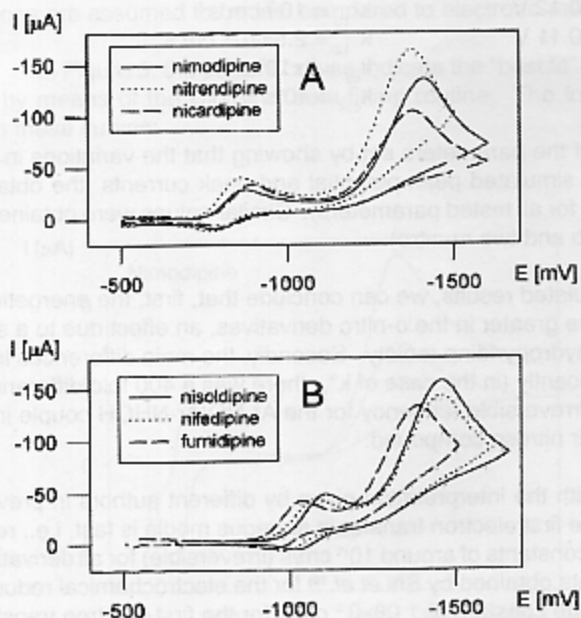
FIG. 4. Experimental CV curves obtained from 1 mM solution of all nitroaryl 1,4-dihydropyridines in mixed media; 40:60/Citrate buffer (pH = 9.0):DMF. Sweep rate 1 V/s. (A) meta-nitro derivatives and (B) ortho-nitro derivatives.

In order to obtain the parameters to simulate the CV responses of the reduction in this media, we systematically varied the set parameters to arrive at the best fit by: (i) visually observing the effect of these changes on deviations between the simulated and experimental CV curves, and (ii) making sure that the final optimized set accurately simulated the CV curves as a function of the nitro derivative concentration and scan rate.

In Figure 5, we can observe the "best-fit" obtained from the experimental CV (Figure 4). The following parameters were used in order to obtain this fit.

Nifedipine:
(o-nitro)

$E^°_5 = -1.09$
$E^°_7 = -0.87$ V
$E^°_{10} = -0.60$ V
$E^°_{12} = -0.53$ V

$k^°_5 = 2.45$ cm s^{-1}
$k^°_7 = 5.4 \times 10^{-5}$ cm s^{-1}
$k^°_{10} = 3.4 \times 10^{-2}$ cm s^{-1}
$k^°_{11} = 0.1$ cm s^{-1}
$k_{c9} = 5 \times 10^2$ s^{-1}
$k^{c15} = 4.12 \times 10^3$ M^{-1}s^{-1}

Nitrendipine:
(m-nitro)

$E^°_5 = -0.91$ V
$E^°_7 = -0.79$ V
$E^°_{10} = -0.55$ V
$E^°_{12} = -0.5$ V

$k^°_5 = 1.37$ cm s^{-1}
$k^°_7 = 1.2 \times 10^{-5}$ cm s^{-1}
$k^°_{10} = 1$ cm s^{-1}
$k^°_{12} = 1$ cm s^{-1}
$k_{c9} = 10^3$ s^{-1}
$k_{c15} = 4.55 \times 10^3$ M^{-1} s^{-1}

FIG. 5. Simulated CV curves obtained from 1 mM solution of Nifedipine and Nitrendipine in mixed media; 40:60/Citrate buffer (pH = 9.0):DMF. Sweep rate 1 V/s.
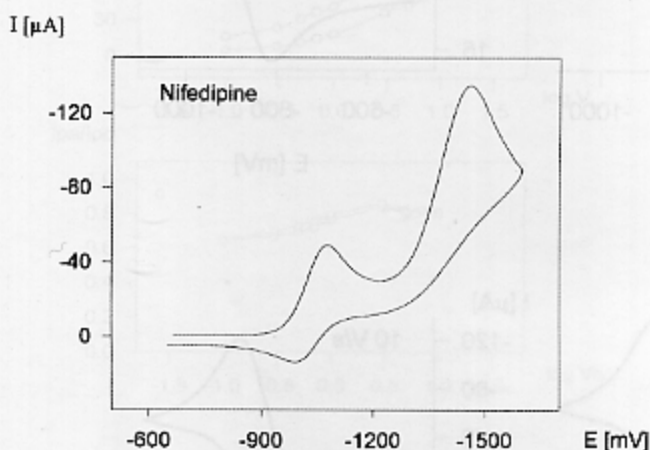
In this media, we observed that there also exists a difference between o-nitro and m-nitro derivatives with respect to: a) the energetics of the electron transfer (E° values) and b) the irreversibility of the Ar-NO/Ar-NHOH couple, which are greater for o-nitro. On the other hand, the heterogeneous rate constant for the first electron transfer ($k°_s$) is similar for both derivatives and is greater than in the protic media.

The above parameters provide an explanation for the splitting phenomenon when aprotic solvents or inhibitors are added. The first important change is that the rate of the first electron transfer step is increased, becoming reversible, i.e., $k°_s \sim 2$ cm/s. The second relevant change is an increase in the reduction potentials (in a cathodic direction) of all the electron transfer steps and the last important change is the addition of the dismutation step 15 with a $k_{c15} \sim 4300$ M^{-1}s^{-1}, which was experimentally obtained by cyclic voltammetry[20] using the Olmstead theory[21].

Some simulated cyclic voltammograms are shown in Figure 6. They were acquired for the isolated couple $ArNO_2/ArNO_2^-$ as a function of the scan rate. The simulation results are qualitatively consistent with the experimental results obtained in the 5 mM Nitrendipine solution in mixed media. Figure 7a,b and c shows the dependence of peak potentials E_{pa}, E_{pc}; the peak currents i_{pa}, i_{pc} and the peak current ratio i_{pa}/i_{pc} for the simulated and experimental results as a function of the potential scan rate. It can be seen that there is reasonably good agreement in all cases between simulated and experimental results.
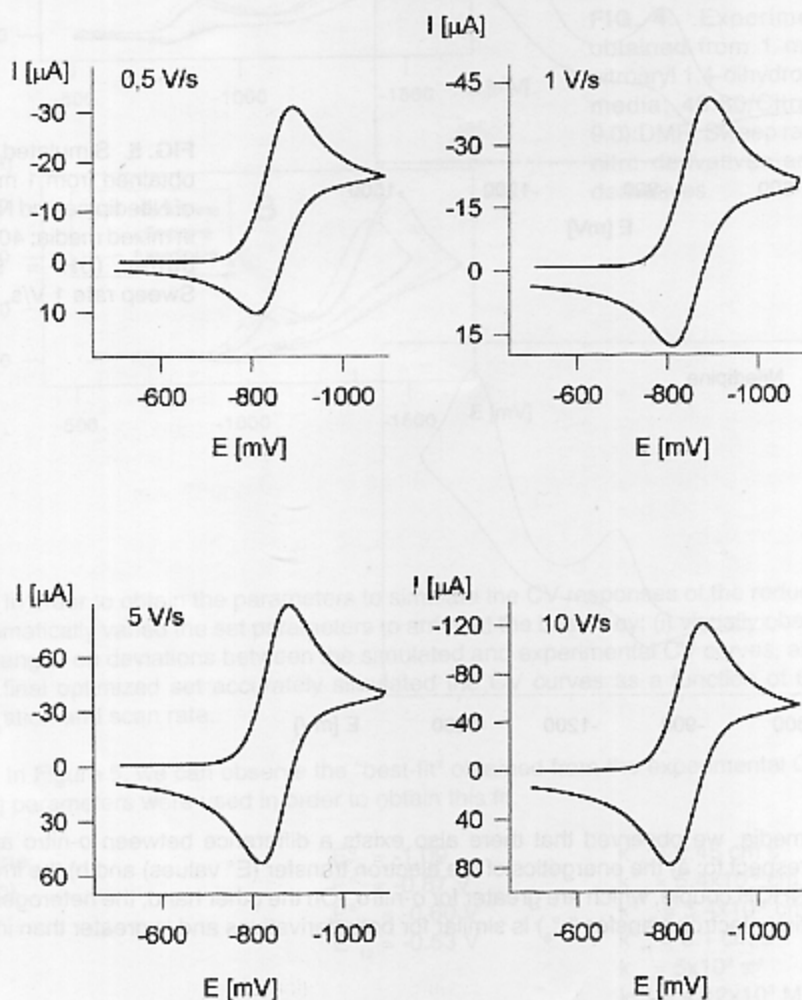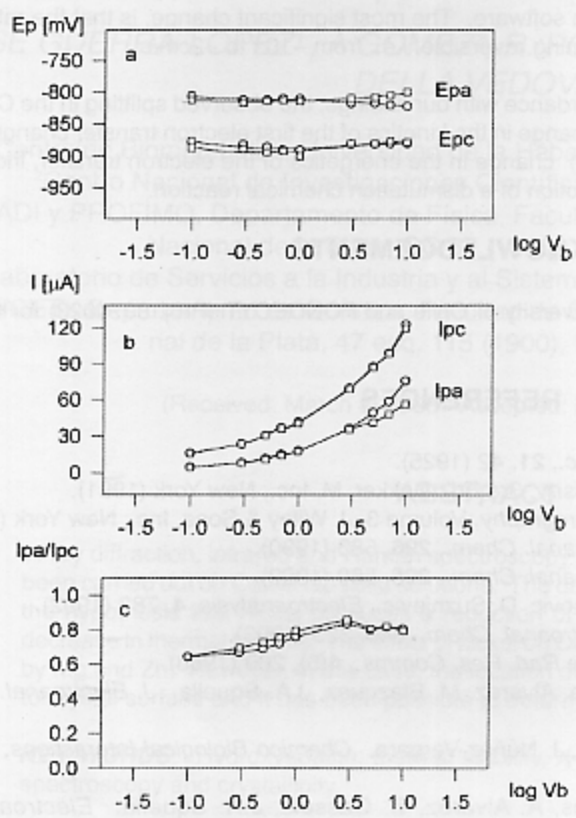

FIG. 6. Simulated CV curves obtained from 5 mM solution of Nitrendipine in mixed media: 40:60/Citrate buffer (pH = 9.0):DMF as a function of scan rate.

Ep [mV]

-750
-800   Epa
-850
-900   Epc
-950

a

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5   log V_b

I [µA]

120   Ipc
90
60   Ipa
30
0

b

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5   log V_b

Ipa/Ipc

1.0
0.8
0.6
0.4
0.2
0.0

c

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5   log V_b

FIG. 7. Dependence of experimental simulated values of: a) peak potentials, E_{pa} and E_{pc}; b) peak currents, i_{pa} and i_{pc}; and c) peak current ratios, i_{pa}/i_{pc}, on potential scan rates obtained from the couple of 1 mM Nimodipine in mixed media. Simulated (O) and (□) experimental values.

CONCLUSIONS

The agreement of the simulated and experimental current is good, especially in the important features of the voltammogram which contain all the information about the mechanism (the wave shapes, peak potentials and peak currents). The electrochemical reduction of nitro aromatic compounds is a rather complex process due to the dependence among the redox pathways, the reaction media and the position of the nitro group in the aromatic molecule.

In the case of the position of the nitro substitution in the molecule, it is clear that the o-nitro, nifedipine, nisoldipine and furnidipine, present more negative formal electrode potentials of about 100 mV. Moreover, they also present values for the electron transfer in reactions 10 and 12, lower than the m-nitro, indicating a great irreversibility in the couple Ar-NO/Ar-NHOH for these compounds in both media.

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When the reaction media is changed, it is clear that the splitting phenomenon is produced in our case, by adding an aprotic solvent such as DMF to the aqueous solution. The splitting is properly explained by a change in the electron transfer parameters, i.e., reduction potentials ($E°$), heterogeneous rate constants ($k°_s$) and the addition of the dismutation step 15. All the above parameters, $k°$ and $E°$, were results obtained with the simulation software. The most significant change, is that the rate of the first electron transfer is increased, becoming reversible, i.e., from $\sim10^{-5}$ to ~2cm/s.

We finally conclude that in accordance with our findings, the observed splitting in the CV curve of nitroaromatics is mainly due to: a) a change in the kinetics of the first electron transfer changing from an irreversible to a reversible process; b) change in the energetics of the electron transfer, increasing the reduction difficulty and c) the introduction of a dismutation chemical reaction.

ACKNOWLEDGEMENTS

REFERENCES

1.   M. Shikata. *Trans. Faraday Soc.*, **21**, 42 (1925).
2.   H. Lund. *Organic Electrochemistry*, 3rd Ed.: Dekker, M. Inc., New York (1991).
3.   B. Kastening. *Progress in Polarography*, Volume 3, J. Wiley & Sons, Inc., New York (1972).
4.   P. Zuman, Z. Fijalek. *J. Electroanal. Chem.*, **296**, 583 (1990).
5.   P. Zuman, Z. Fijalek. *J. Electroanal. Chem.*, **296**, 580 (1990).
6.   P. Zuman, Z. Fijalek, D. Dumanovic, D. Suznjevic. *Electroanalysis*, **4**, 783 (1992).
7.   A. Kalandyk, J. Stroka. *J. Electroanal. Chem.*, **346**, 323 (1993).
8.   J.H. Tocher, D.I. Edwards. *Free Rad. Res. Comms.*, **4**(5), 269 (1988).
9.   L.J. Núñez-Vergara, S. Bollo, a. Alvarez, M. Blazquez, J.A. Squella. *J. Electroanal. Chem.*, **345**(1,2), 121 (1993).
10.   J.A. Squella, C. Solabarrieta, L.J. Núñez-Vergara. *Chemico Biological Interactions*, 89, 197 (1993).
11.   L.J. Núñez-Vergara, C. Matus, A. Alvarez, B. Cassels, J.A. Squella. *Electroanalysis*, **7**(5-6), 509 (1994).
12.   M. Rudolph, D.P. Reddy, S. Feldberg. *Anal. Chem.*, **66**, 589A (1994).
13.   E.G. Jäeger, M. Rudolph. *J. Electroanal. Chem.*, **434**, 1 (1997).
14.   M. Rudolph, S.W. Feldberg. *Digisim 2.0 Software* 1995 Bioanalytical System Inc., West Lafayette, IN 47906 USA.
15.   D.K. Gosser. *Cyclic Voltammetry Simulation and Analysis of Reaction Mechanisms* 1993 V.C.H. Publishers, Inc., New York 91.
16.   C. Shi, W. Zhang, R.L. Birke, D.K. Gosser Jr., J.R. Lombardi. *J. Phys. Chem.*, **95**, 6276 (1991).
17.   P. Gao, D. Gorsztola, J. Weaver. *J. Phys. Chem.*, **92**, 7122 (1988).
18.   J.A. Squella, S. Bollo, J. de la Fuente, L.J. Núñez-Vergara. *Bioelectrochem. Bioenerg.*, **34**(1), 13 (1994).
19.   L.J. Núñez-Vergara, F. García, M. Domínguez, J. de la Fuente, J.A. Squella. *J. lectroanal. Chem.*, **381**, 215 (1995).
20.   L.J. Núñez-Vergara, M.E. Ortiz, S. Bollo, J.A. Squella. *Chemico Biological Interactions*, **106**, 1 (1997).
21.   M.L. Olmstead, R.G. Hamilton, R.S. Nicholson. *Anal. Chem.*, **41**, 260 (1969).