# Electrolytic Oxidation of C4-Nitrofuryl 1,4-Dihydropyridines in Nonaqueous Medium

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A study of the electrolytic oxidation of three new C-4 nitrofuryl 1,4-dihydropyridines in nonaqueous aprotic medium is presented. Controlled-potential electrolysis (CPE) in dimethylformamide +0.1 M tetrabutyl-ammonium hexafluorophosphate ($TBAPF_6$) was followed by UV-visible spectroscopy, high-performance liquid chromatography (HPLC)-photodiode array (PDA), and gas chromatography-Mössbauer spectroscopy (GC-MS) chromatography, and electron spin resonance and electrochemical techniques. Carbon-centered radical intermediates produced in the electrochemical oxidation of C-4 nitrofuryl substituted 1,4-dihydropyridines were trapped with N-benzylidene-*tert*-butylamine-N-oxide (PBN) and their splitting constants were calculated. The neutral pyridine derivatives were identified by GC-MS techniques as final oxidation products. HPLC-PDA and GC-MS chromatographic techniques were used to follow the time-course of CPE of both parent 1,4-DHP derivatives and their respective oxidation products. Also, an overall oxidation mechanism of C-4 nitrofuryl-1,4-DHP derivatives is presented.

Dihydropyridine drugs (1,4-DHPs), such as nifedipine, nicardipine, amlodipine and others, are clinically effective cardiovascular agents for the treatment of hypertension and have been intensively studied to elucidate the molecular and conformational requirements for their attractive calcium antagonist activities.[1,2]

Some short-action dihydropyridine drugs must be used with caution in patients with coronary diseases because of the increased mortality observed in patients treated with these drugs.[3-5] To resolve this and other types of problems, the synthesis of new 1,4-dihydropyridines was focused in different directions.[6-8] Thus, the chemical incorporation of a dihydropyridine-type calcium channel blocker and α/β adrenoceptor blocker molecule displaying these three blockades[9] has been used as one of the strategies to avoid the above-mentioned undesirable effects. On the other hand, to date, the structure-activity relationship of the dihydropyridines indicated that most of the desired properties rely on the structural characteristics of the substituent at the 4-position of the dihydropyridine nucleus and especially if that substituent is an aromatic phenyl ring. Modification of this phenyl ring on the dihydropyridine compounds demonstrated that the introduction of electron-donating substituents and electron-withdrawing substituents produced relevant changes in the pharmacological effects.[10] 1,4-DHPs exhibit other additional effects which are not directly associated with the calcium-channel antagonist effects, among which the following can be mentioned: antioxidant effects,[11-15] the release of nitric oxide,[16,17] and trypanocidal actions.[18-20]

Oxidation of 1,4-DHPs and analogues to the corresponding pyridines is interesting because of its relevance to the biological dihydronicotinamide adenine dinucleotide (NADH) redox process.[21,22] Investigations dealing with the electrochemical oxidation of some 1,4-DHPs have previously been performed in both nonaqueous[23-26] and aqueous media.[27-29] We recently reported[30] the synthesis and electrochemical oxidation of some new 3,5-(substituted)-4-(5′-nitro-2′-furyl)-1,4-DHP derivatives in a protic medium. In that paper, a detailed study in aqueous medium on the electro-oxidation of these compounds is reported. In acidic medium at pH $< 4$ the oxidation mechanism of compounds obeyed the electrochemical-chemical (ECE) sequence. However, at pH $> 4$ the derivatives follow an ECEC sequence. In this paper, the electrolytic oxidation in aprotic medium of the mentioned 1,4-DHPs is studied. These compounds were synthesized having in mind the development of new structures with mixed effects, i.e., calcium-channel antagonism and trypanocidal effects. Finally, we report the identification of intermediates and the final products of the electrolytic oxidation. On the basis of this information, a tentative oxidation mechanism is proposed.

## Experimental

*Chemicals*.— All solvents were of high-performance liquid chromatography (HPLC) grade and all reagents were of analytical grade.

*Compounds*.— All nitrofuryl 1,4-dihydropyridine derivatives (Fig. 1) were synthesized in our laboratory according to previous work.[30]

*Ethyl 4-(2′-furyl-5′-nitro)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (Compound I)*.— Yield: 60%. mp 184.2°C. IR (KBr): ν max 3346.7, 1704.3, 1656.0, 1517, 1487.9, 1400, 1355.0, 1211.9, 1114.4. $^1$H NMR (300 MHz, $CDCl_3$): δ 1.28 (dd, 6H, $J$ = 7.1, $J$ = 7.1); 2.36 (s, 6 H); 4.15 (dq, 2H, $J$ = 10.9, J = 7.1); 4.21 (dq, 2H, $J$ = 10.9, $J$ = 7.1); 5.27 (s, 1H); 6.19 (br s, 1H); 6.26 (d, 1H, $J$ = 3.4), 7.21 (d, 1H, $J$ = 3.4). $^{13}$C NMR (75 MHz, $CDCl_3$): 166.8 (C2), 162.7 (C2), 146.4 (C2), 113.2, 108.9, 99.0 (C2), 60.2 (C2), 34.6, 19.6 (C2), 14.3 (C2) ppm. Anal. Calcd. for $C_{17}H_{20}N_2O_7$: C, 56.04; H, 5.53; N, 7.69. Found: C, 55.85; H, 5.54; N, 7.72.

*3,5-dicyano—4-(2′-furyl-5′-nitro)-2,6-dimethyl-1,4-dihydropyridine (Compound II)*.— Yield: 43%. mp 225.2°C. IR (KBr): ν max 3369.2, 2205.9, 1665.8, 1536.3, 1505.1, 1393.1, 1358.8. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 2.07 (s, 6 H); 4.92 (s, 1H); 9.81 (br s, 1H); 6.82 (d, 1H, $J$ = 3.8), 7.70 (d, 1H, $J$ = 3.8). $^{13}$C NMR (75 MHz, DMSO-$d_6$): 159.7 (C2), 158.0 (2), 121.8 (C2), 114.9, 111.9, 73.8 (C2), 33.9, 20.8 (C2) ppm. Anal. Calcd. for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73. Found: C, 57.75; H, 3.73; N, 20.65.

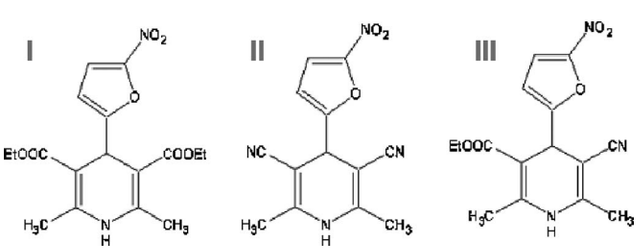


**Figure 1.** Chemical structures of the nitrofuryl 1,4-DHP compounds.

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*Ethyl 3-cyano-4-(2′-furyl-5′-nitro)-2,6-dimethyl-1,4-dihydropyridine-5-carboxylate (Compound III).*— Yield: 80%. mp 161.9°C. IR (KBr): $\nu$ max 3317.9, 2199.4, 1664.3, 1638.5, 1527.1, 1493.1, 1383.0, 1355.5, 1257.0, 1115.7. $^1$H NMR (300 MHz, $CDCl_3$): δ 1.29 (dd, 3H, *J* = 7.1, *J* = 7.2); 2.18 (s, 3 H); 2.38 (s, 3 H); 4.12 (dq, 1H, *J* = 10.9, *J* = 7.1); 4.16 (dq, 1H, *J* = 10.9, *J* = 7.2); 4.90 (s, 1H); 6.04 (br s, 1H); 6.38 (d, 1H, *J* = 3.7), 7.24 (d, 1H, *J* = 3.7). $^{13}$C NMR (75 MHz, $CDCl_3$): 166.2, 160.0 (C2), 147.6, 146.3, 118.6, 113.0, 109.5, 97.6, 81.8, 60.5, 35.9, 19.7, 18.5, 14.2 ppm. Anal. Calcd. for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.56; H, 4.77; N, 13.19.

*Electrolytic media.*— All compounds were dissolved in dimethylformamide (DMF) containing 0.1 M tetrabutylammonium hexafluoro phosphate ($TBAPF_6$) to obtain final concentrations varying between 0.05 and 5.0 mM. A routine concentration of 1 mM of 1,4-DHP derivatives was used. Solutions were bubbled with extra pure nitrogen (Indura, Santiago, Chile, $H_2O < 3$ ppm, $O_2 < 2$ ppm $H_nH_m < 0.5$ ppm) before each determination.

*Voltammetry.*— Both differential pulse voltammetric (DPV) and cyclic voltammetric (CV) measurements were performed with a BAS CV 100 assembly. Glassy carbon was employed as working electrode. A platinum wire was used as a counter electrode and all potentials were measured against a Ag/AgCl electrode.

*Controlled-potential electrolysis (CPE).*— CPE were carried out at a glassy carbon mesh electrode in DMF containing tetrabutylammonium perchlorate as supporting electrolyte. The applied potentials were 1.40 V (compound I), 1.12 V (compound II), and 1.35 V (compound III). Oxygen was removed with pure and dry presaturated nitrogen. A three-electrode circuit was used with a Ag/AgCl reference electrode and a platinum wire counter electrode. A BAS-CV 100 assembly was used to electrolyze the different derivatives.

*Electron spin resonance (ESR) measurements.*— ESR spectra were recorded in situ on a Bruker spectrometer ECS 106 with 100 -kHz field modulation in X band (9.78 GHz) at room temperature. Hyperfine splitting constants were estimated to be accurate within ±0.05 G. The electrolysis was performed under the same conditions as described above. The concentration of the spin trap, N-benzylidene-t-butylamine-N-oxide (PBN), was at least 100 times higher to ensure the trapping of the radical species. This molecule was electrochemically tested for purity and the trap is electrochemically inactive. The oxidation peak potential of PBN in DMF + 0.1 M $TBAPF_6$ at a Pt electrode was +1.43 V vs Ag/AgCl.

*UV-visible spectroscopy.*— Spectra were recorded with a UNICAM UV-3 spectrophotometer. UV-visible spectra were recorded in the 200–400 nm range at different intervals. Acquisition and data treatment were carried out with Vision 2.11 software.

*High performance liquid chromatography (HPLC) analysis on the electrochemical oxidation of 1,4-DHPs.*— HPLC measurement were carried out with the aid of a Waters assembly equipped with a model 600 controller pump and a model 996 photodiode array detector. The acquisition and treatment of data were done by means of the Millennium version 2.1 software. As chromatographic column, a μBondapak/μPorasil C-18 column (3.9 × 150 mm) was used. A C-18 μBondapak precolumn (30 × 4.6 mm) was employed. The injector was a 20 μL Rheodyne valve. An aliquot of the electrolyzed solution of each 1,4-dihydropyridine was taken and a 20 μL volume of these solutions was injected into the chromatographic system. A mixture of methanol and 0.05 M phosphate buffer (55/45) at pH 4.3 was used as mobile phase.

The photodiode array (PDA) detector operated at 250 nm for quantitation. The flow of mobile phase was maintained at 1 mL/min and a helium bubbling of 30 mL/min was applied to remove dissolved gases. For the determination of the analytical parameters described below, an injection volume of 20 μL was used.

**Table I. Electrochemical and spectral characteristics of parent nitrofuryl 1,4-DHP and their corresponding oxidation products.**

| Compound | Peak potential[a] /mV | $\lambda_{max}$[b] 1,4-DHP | $\lambda_{max}$[b] Pryidine |
|---|---|---|---|
| I | 1304 | 333 | 273 |
| II | 1016 | 319 | 271 |
| III | 1248 | 322 | 270 |

[a] Average of oxidation peak potentials obtained by differential pulse voltammetry in aprotic media.
[b] Spectral characteristics obtained from UV-visible measurement.

*GC-MS.*— A gas chromatograph-mass selective Hewlett-Packard 5890/5972 detector (Palo Alto, California, USA) and Hewlett-Packard 7673 autosampler were used for the measurements. A Hewlett-Packard Pentium II Data System controlled instrumentation and data handling.

*Chromatography column.*— Hewlett-Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11 film thickness (Little Falls, Wilmington, DE, USA).

*Chromatographic conditions.*— Detector temperature, 300°C; injector temperature, 250°C; split ratio, 1/10; pressure, 13 psi; purge flow, 40 mL min$^{-1}$; purge time, 0.5 mL min$^{-1}$.

*Temperature program.*— The oven temperature was programmed from 130 to 305°C (hold for 5 min) at 15°C min$^{-1}$; run time was 16.67 min. Helium was used as carrier gas with an inlet pressure of 35 kPa. The identification of the samples was based on the analyses of the mass spectra (full scan).

## Results and Discussion

The aim of this work was to examine the electrolytic oxidation in a nonaqueous medium of a series of three synthesized C-4 nitrofuryl-1,4-DHP derivatives (Fig. 1). We have focused our efforts towards the identification of the intermediate and the final product after the electrolytic oxidation of each compound by using electrochemical, spectroscopic, and chromatographic techniques.

*Electrochemistry.*— Cyclic voltammetry (CV) of the C-4 nitrofuryl-1,4-DHPs on a glassy carbon electrode revealed an irreversible anodic signal at sweep rates varying between 0.1 and 10 V s$^{-1}$. In all cases, log $I_p$ vs log $\upsilon$ plots exhibited slopes close to 0.5, indicating that no adsorption is involved in the oxidation process. Furthermore, the peak potentials ($E_p$) were dependent on the sweep rate with $\delta E_p/\delta \log \upsilon$ values of 39.5, 29.2, and 32.1 for compounds I–III, respectively. To determine the number of electrons transferred in the oxidation process in the aprotic medium studied, coulometric analysis was carried out. Solutions containing accurately weighed amounts of the 1,4-DHPs were subjected to electrolysis at constant applied potential and the charge passed was registered. From these experiments it was confirmed that, in this medium, compounds I–III are oxidized according to a two-electron overall process (experimental average of electrons transferred = 2.05 ± 0.02 for all the compounds).

To study the time-course of the controlled-potential electrolysis (CPE) experiments, the intermediate species, and the formation of the final oxidation products, we used CV and DPV techniques. Through these latter techniques, the following order of oxidation easiness was determined: II > III > I (Table I). This result is in agreement with the electron-withdrawing substituents at the 3- and 5-positions on the dihydropyridine ring.

In Fig. 2, the time-course of a CPE of a 1 mM concentration of compound I followed by its peak current (Fig. 2a, DPV technique) and CV (Fig. 2b) are shown. As can be seen in Fig. 2a, the peak

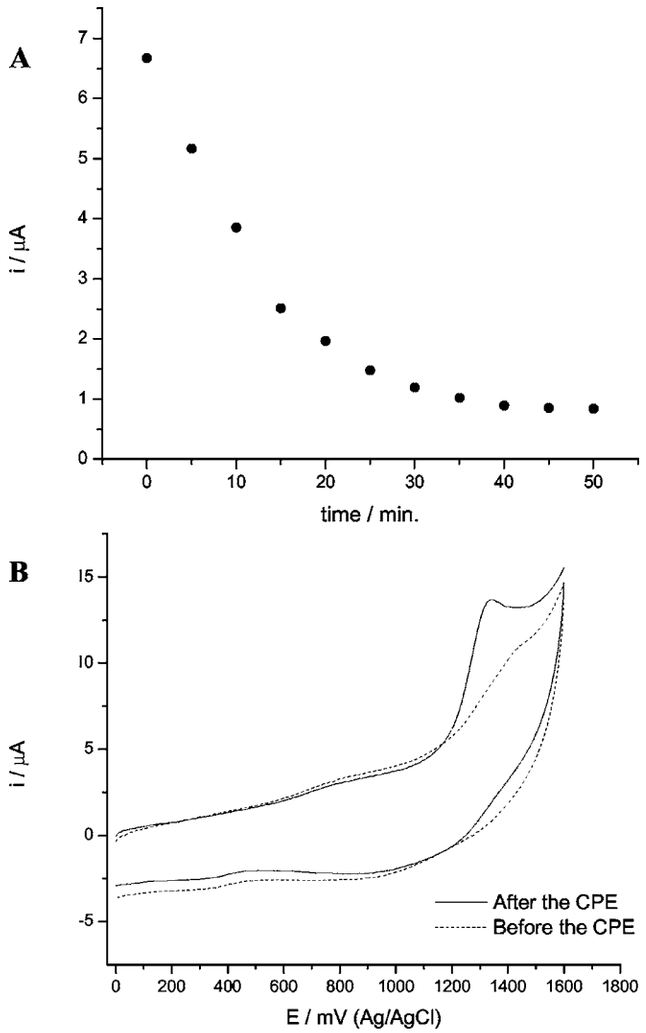



**Figure 2.** Time-course of CPE of 1 mM compound I in dimethylformamide +0.1 M $TBAPF_6$ followed by DPV on a glassy carbon electrode. I–VII: 38 min. Insert: Time-course of peak current.

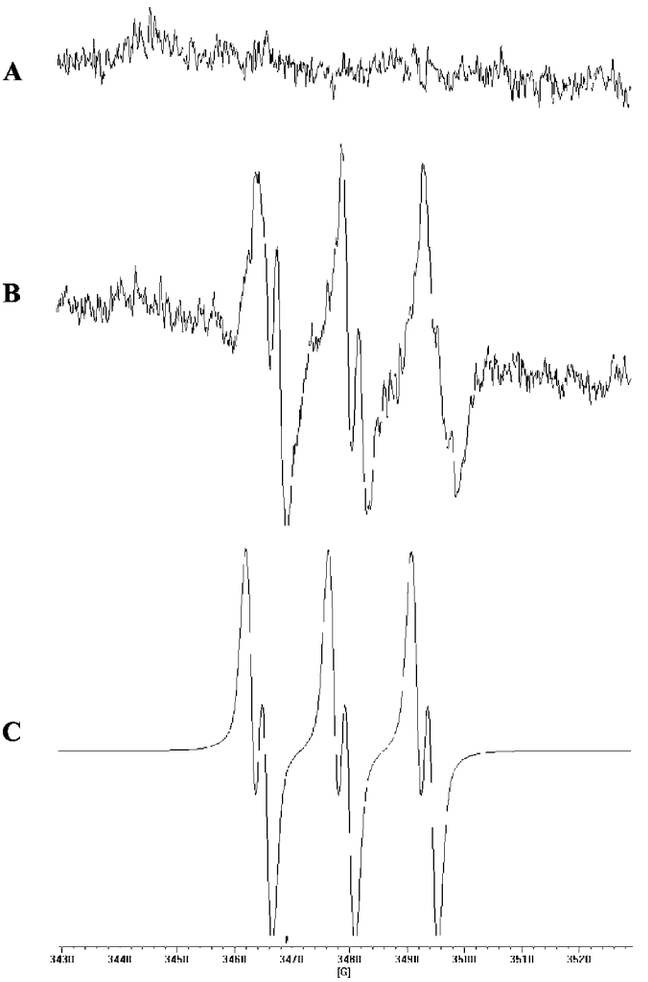
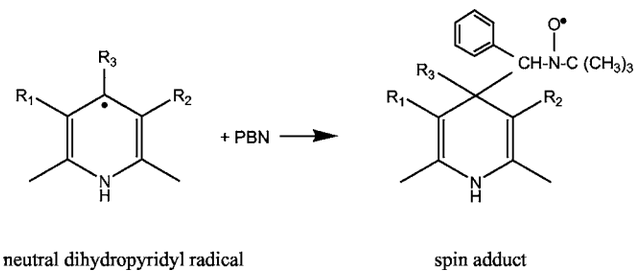



**Figure 3.** ESR spectra of: A: base line, B: spin adduct of the in situ electrooxidation of 1 mM compound I in the presence of 0.1 M PBN in dimethylformamide +0.1 $TBAPF_6$. C: Simulated spectrum of compound I.

current diminished with the time of electrolysis. On the other hand, in Fig. 2b CVs before and after the electrolysis are shown. The expected decrease of current was observed, but the appearance of any new signal (intermediates or final products) in the potential range studied was not noted. In addition, after 50 min of electrolysis, practically all the original compound was consumed. Similar results were obtained for compounds II and III. In conclusion, this technique proved to be inadequate to follow the appearance of either intermediate species or final oxidation products. Moreover, the further reduction of products formed during the oxidation process could not be detected with the different working electrodes (Hg or glassy carbon electrodes) tested.

*ESR characterization of free radicals generated from nitrofuryl 1,4-DHPs.*— To identify the intermediates produced in the time-course of the electrolysis of compounds I–III, spin trapping studies were conducted. The experimental ESR spectra show a triplet, due to the nitrogen, and its splitting into a doublet due to the presence of the adjacent hydrogen. The corresponding $a_N$ values were: 14.7 G, 14.3 G, and 14.4 G for compounds I, II, and III, respectively. On the other hand, $a_H$ splitting values for the same compounds were: 3.04 G, 2.40 G, and 2.60 G, respectively. The above splitting constants for the spin adducts are consistent with the fact that PBN interacted with carbon-centered radicals as reported for 1,4-DHP derivatives[31] and for other structurally-related compounds.[32] Experimental and simulated ESR spectra for the adduct derived from compound I are shown in Fig. 3. The experimental ESR spectrum (Fig. 3b) shows the above-described general characteristics. Likewise, the simulated spectrum (Fig. 3c) is in good agreement with the experimental one. In previous work[27,33] on the electro-oxidation of 1,4-DHP derivatives, the formation of different types of radical intermediates was discussed. Primarily, a radical cation $PyH^{+\bullet}$ is formed and, after its deprotonation, a neutral radical $Py^{\bullet}$ (dihydropyridyl radical) is formed. Considering the present ESR results, it seems likely that the species added to the PBN spin trap is a dihydropyridyl radical. From previous quantum chemical calculations,[34] it follows that the unsubstituted pyridyl radical, $Py^{\bullet}$, which has the unpaired electron density in positions 2-, 4-, or 6-, with the highest electron density at the 4-position[34] seems to be the preferred one. Taking into account the above-described information and our experimental data on hyperfine splitting constants, Scheme 1 is proposed for the trapping of neutral dihydropyridyl radicals by PBN. In addition, electron-acceptor substituents at the 3- and 5-position help to increase the stability of the radicals. Such effects are additive in the 4-position, leading to an even greater preference for this position. Consequently, one may assume that the radicals generated in our experiments are preferentially added to the spin trap by this C-4 reactive position according to Scheme 1.




**Scheme 1.** Proposed reaction for the trapping of radical species by PBN generated during the controlled-potential electrolysis of the C-4 nitrofuryl DHPs.

*UV-visible spectroscopy.*— The three C-4 nitrofuryl-1,4-DHPs exhibit a single well-defined UV-visible absorption band at 333 nm (compound I), 321 nm (compound II), and 326 nm (compound III). However, after the electrolytic procedure (CPE), when an anode potential close to the oxidation peak was applied, these bands diminished in intensity in parallel with the appearance of new bands between 270–285 nm, depending on the compound. According to previous work[35,36] the new above-described UV absorption bands would correspond to the pyridine derivative formation. In Fig. 4, the UV-visible differential spectra for the time-course of the CPE of compound I are shown. Thus, the original UV band at 333 nm decreased with electrolysis and the appearance of a new band at 275 nm is observed. The assumption that UV bands in the 270–285 nm correspond to the pyridine derivative formation was confirmed with the identification of the compound by GC-MS techniques.

*HPLC-PDA characterization of the electrochemical oxidation of nitrofuryl 1,4-DHPs.*— For these studies the following mobile phase was selected from preliminary experiments: 50 mM buffer phosphate-methanol (55/45) at pH 4.3. The relative standard deviation of the retention time was $<1\%$, thus indicating high stability of the system. On the other hand, linearity occurred at least from 0.01 to 0.2 mM (AUC = $-163725.5 + 4.9527 \times 10^{10}$ (M), ($R^2 = 0.99989$). The reproducibility was 1.9%, the detection limit was $2.1 \times 10^{-6}$ M.

In Fig. 5a, the time-course of CPE followed with HPLC-PDA for compound III is displayed. As can be seen, the main signal corresponding to the parent 1,4-DHP appears at zero and short times of electrolysis; but, from 6 min of electrolysis, a second signal corresponding to the oxidation product was evidenced. In the insert of Fig. 5b, the evolution of the area under the curve (AUC) corresponding to the parent compound II and its pyridine derivative are shown. Similar behavior was found for the other compounds. In conclusion, the main advantage of this technique resides in the simultaneous quantitative determination of both the parent nitrofuryl 1,4-DHP derivative and its oxidation product generated in the course of the bulk electrolysis.

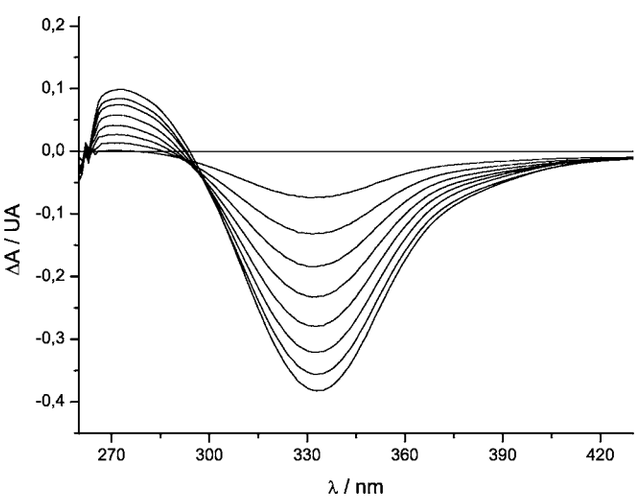



**Figure 4.** UV-visible differential spectra of the time-course of CPE corresponding to 0.1 mM compound I.

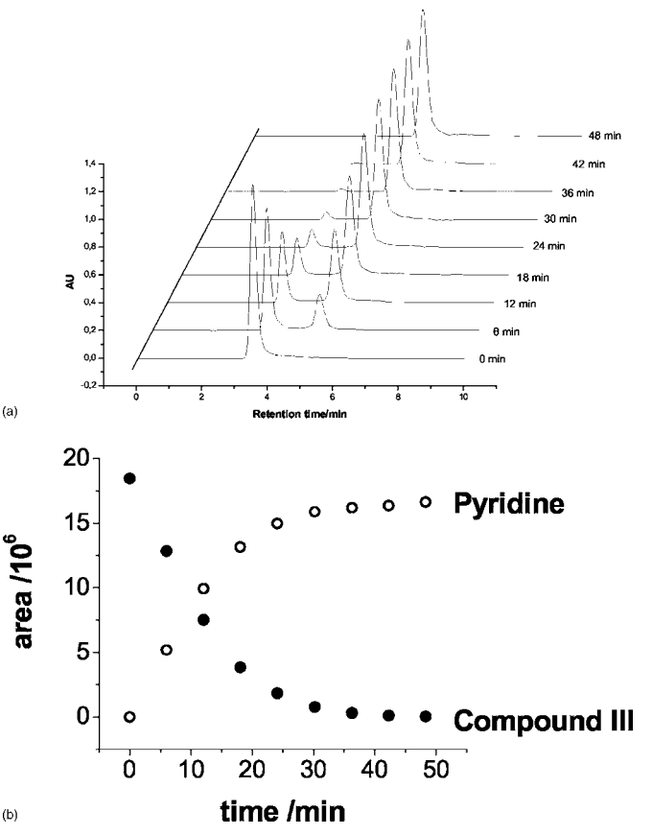



**Figure 5.** (A) Time-course of CPE of 1 mM compound III followed by HPLC—PDA during 48 min. (B) Time-course of the area under curve of CPE followed by HPLC-PDA: (●) peak at 3.57 min (parent compound III) and (○) peak at 5.17 min (pyridine).

*GC-MS studies.*— These studies were developed with two main goals, to identify the final oxidation products and to follow the time-course of the controlled-potential electrolysis of the derivatives. The main fragmentation of compound I is the cleavage of the ethoxycarbonyl branch at 3-position $[M\text{-}73]^+$. However, both cyano-substituted DHPs (compound II and II) are characterized by the cleavage of the nitrofuryl group formerly in 4-position $[M\text{-}112]^+$. The pyridine derivatives exhibit a different fragmentation pattern, thus, compound II suffers no fragmentation under the experimental conditions. The pyridine derivative from compound I experiments the cleavage of both ethoxycarbonyl groups at 3- and 5-positions of the DHP ring $[M\text{-}146]^+$. Finally, compound III lost the ethoxycarbonyl group at the 5-position.

In Fig. 6 and 7 the ion chromatogram and mass spectrum corresponding to the parent compound III before and after the CPE are shown. As can be seen from Fig. 6, *m/z* for the molecular ion $M^+$ was 317 and the base peak of parent compound III corresponded to the complete loss of the C-4 substituent $[M\text{-}112]^+$. In Fig. 7 it can be seen that *m/z* for the molecular ion was 315 and the base peak of

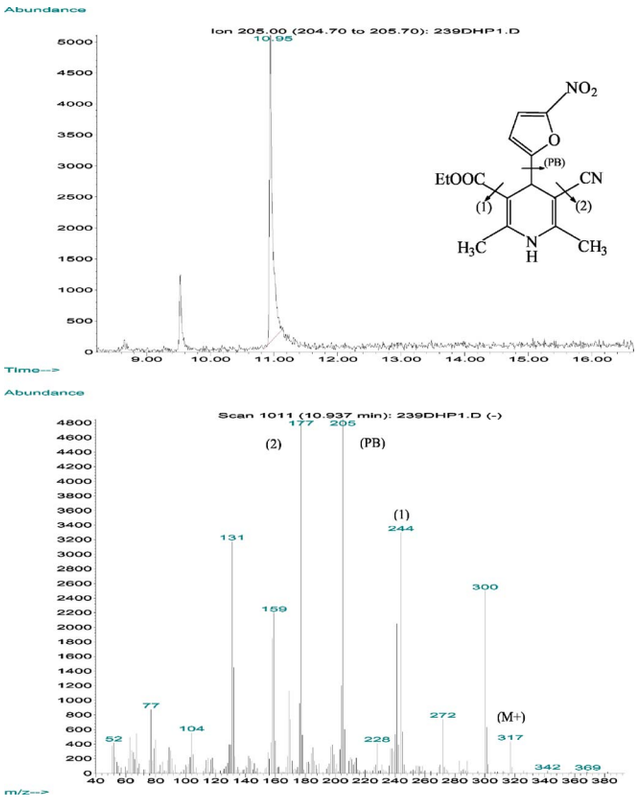



**Figure 6.** (Color online) Ion chromatogram and mass fragmentation corresponding to parent compound I.

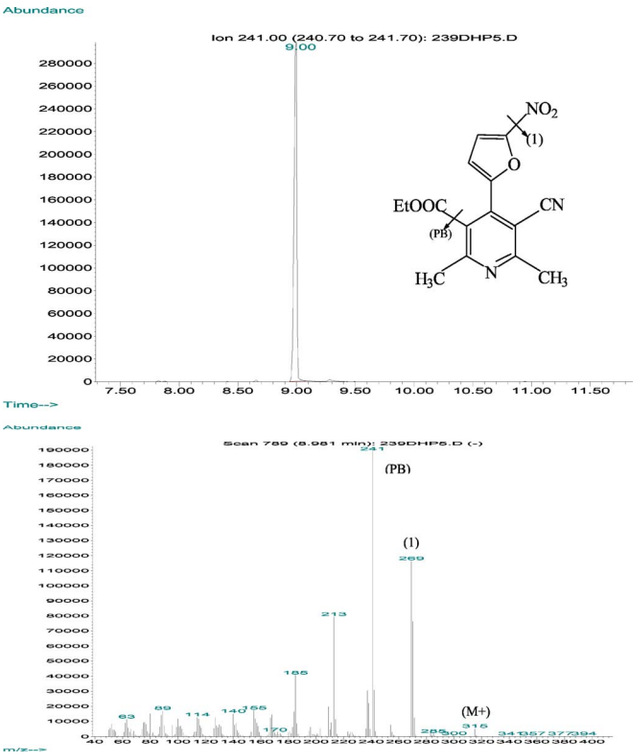



**Figure 7.** (Color online) Ion chromatogram and mass fragmentation corresponding to the oxidized compound I after the electrolysis (60 min).

the pyridine derivative (oxidized compound) corresponded to the complete loss of the 3-position substituent $[M\text{-}74]^+$.

These results confirmed that, after the electrochemical oxidation under the experimental conditions reported here, the C-4 nitrofuryl 1,4-DHPs generated the pyridine derivatives as the major oxidation products.

In Fig. 8 the time-course of a CPE of compound III followed by the base peaks of parent and pyridine derivatives is displayed. As expected, in parallel with the decrease of % abundance of base peak corresponding to the parent compound III (*m*/*z* 205), a significant increase in the % abundance of base peak of pyridine derivative ( *m*/*z* 241) was observed. These experiments were extended to compounds I and II, with similar results being obtained. In consequence, from these results we conclude that the GC-MS technique is suitable to follow the time-course of the CPE for this type of derivative.

On the other hand, the aromatization of the 1,4-dihydropyridine ring to the corresponding pyridine derivative constitutes the most important in vivo reaction to terminate the pharmacological effect in the current clinically used drugs.[37]

Finally, on the basis of findings presented here and other previous studies[27,29,31,33,34] Scheme 2 concerning the oxidation mechanism in an aprotic medium can be postulated. This mechanism is supported by two experimental facts: (*i*) the generation of the neutral dihydropyridyl radical intermediates C-centered was demonstrated by the trapping of these species with PBN and their characteristic spin adducts were determined by the ESR technique for the three compounds and (*ii*) the pyridine derivatives were identified by GC-MS as the final oxidation products in the aprotic medium.

## Conclusions

We have successfully intercepted the unstable intermediate dihydropyridyl radicals by means of the spin trapping method, using N-benzylidene-t-butylamine-N-oxide (PBN) as the spin trap. Furthermore, the hyperfine splitting constants are consistent with the nature of the radical species, i.e., a neutral dihydropyridyl radical.

The formation of the pyridine derivatives after the electrochemical oxidation of C-4 nitrofuryl 1,4-dihydropyridines was demonstrated by GC-MS identification.

The HPLC-PDA methodology provides a tool which makes possible the simultaneous determination of both the parent 1,4-DHP and their oxidation products, the pyridines.

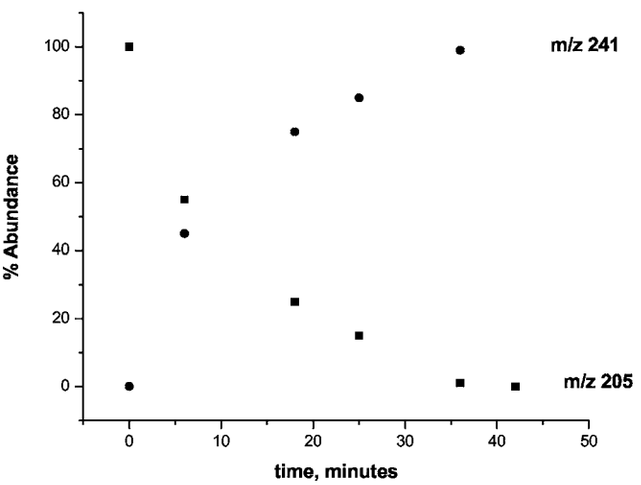
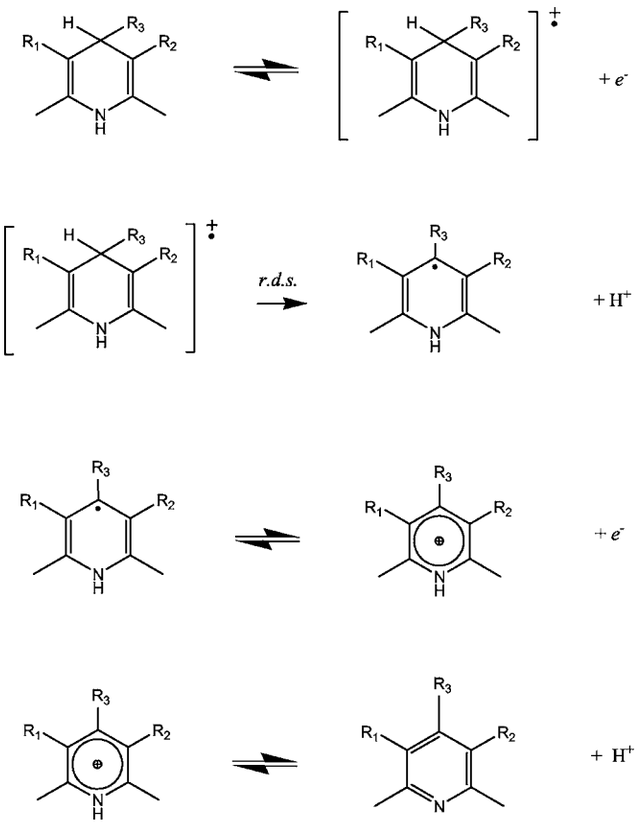



**Figure 8.** Time-course of intensity of *m*/*z* 205 corresponding to the parent compound III and *m*/*z* 241 corresponding to its pyridine analogue after controlled-potential electrolysis.




**Scheme 2.** Overall oxidation mechanism of C-4 nitrofuryl 1,4-DHPs in aprotic medium.

GC-MS also provides an appropriate technique to follow the time-course of the electrolysis of these 1,4-dihydropyridine derivatives.

Scheme 2 presents an overall oxidation mechanism in aprotic medium for the C-4 nitrofuryl 1,4-DHPs.

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