Spectroelectrochemical Study on the Electrooxidation in Aqueous Medium of some 1,4-Dihydropyridines: Effects of Substitution in 1-Position and 4-Position

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Abstract
Spectroelectrochemical and HPLC characterization of the electrochemical oxidation in aqueous medium of a series of six N-1 and C-4 substituted 1,4-dihydropyridines is presented. Based on the analysis of spectra obtained by in situ spectroscopic measurements it was possible to detect the generation of final oxidation products, which resulted in differences depending of the nature of the substitution on the nitrogen in the dihydropyridine ring. Controlled potential electrolysis (CPE) in aqueous medium was followed by the HPLC technique using EC and PDA detectors. This latter resulted adequately to follow the parent 1,4-DHP derivatives and their respective oxidation products. Electrochemical oxidation of parent N-H substituted 1,4-dihydropyridines generated the corresponding neutral pyridine derivative as final oxidation product. However, the *N*-ethyl substituted 1,4-dihydropyridine derivatives gave rise to the pyridinium salt derivatives.

Keywords: Spectroelectrochemical technique, Oxidation, 1,4-Dihydropyridines, Cyclicvoltabsorptogram, HPLC, Pyridine, Pyridinium salt, Controlled potential electrolysis

1. Introduction

4-Aryl-1,4-dihydropyridines (1,4-DHP) have become the most important group in the field of modulation of calcium-evoked contraction. The pharmacological activity of asymmetrically substituted 1,4-DHP depends on the configuration of the stereogenic C-4 carbon atom, which determines whether the compound acts as a calcium antagonist or as a calcium agonist [1–3]. Investigations dealing with the electrochemical oxidation of some 1,4-DHP have previously been performed in both non-aqueous [4–7] and aqueous [8–11] media. In water containing solvents, 1,4-DHP are reported to undergo a simple two-electron anodic process affording the corresponding pyridine derivative and the concomitant release of protons, but deep insight on the mechanistic information has been obtained in non-aqueous solvents, even though major attention has been almost always focused on the kinetics of deprotonation of the cation radical formed as the primary oxidation product. Conversely, some features of the overall oxidation process have led to different interpretations, so that they have remained without reliable explanation as yet. Ludvik et al. [12] proved that 4-disubstituted 1,4-dihydropyridines are electrochemically oxidized in acetonitrile in an EC process, giving rise to an aromatic 4-monosubstituted pyridine or pyridinium cation (in 1-substituted derivatives). Spectroelectrochemical techniques have been extensively used in the determination of a number of parameters [13–15] such as standard potentials, diffusion coefficients, electron transfer rate constants, number of electrons in a electrode reactions, etc. In addition, they have been widely used in the elucidation of reaction mechanisms in different organic, inorganic and biochemical systems [16, 17]. The determination of dihydropyridines in biological fluids has mainly been carried out using gas chromatographic (GC) methods with electron-capture [18, 19], N-P ionization [20] and mass spectrometric detection [21–24] and HPLC with UV [25–27] and electrochemical detection [28]. It is noteworthy, that this latter technique has been applied mainly to the determination of the parent 1,4-dihydropyridines. The present work deals about a systematic spectroelectrochemical study on the oxidation on a glassy carbon electrode of a series of non-commercial N-1 and C-4 substituted 1,4-DHP derivatives in aqueous medium. Furthermore, also we have attempted to develop a simple method to follow the parent 1,4-dihydropyridines and their oxidation products generated during the bulk electrolysis.

2. Experimental

2.1. Chemicals

All solvents were of high-pressure liquid chromatography (HPLC) grade and all reagents were of analytical grade.

2.2. Compounds

All 1,4-dihydropyridine derivatives (Figure 1) were synthesized in our laboratory according to previous works [29, 30].

The final products were analysed by IR, NMR and elemental analyses, obtaining the following results:

(I) 4-methyl-2, 6-dimethyl-3, 5-dimethoxycarbonyl-1,4-dihydropyridine.
(II) 4-(4-methoxyphenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine.
(III) 4-(4-nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine.
(IV) 4-methyl-2,6-dimethyl-3,5-dimethoxycarbonyl-*N*-ethyl-1,4-dihydropyridine.
(V) 4-(4-methoxyphenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-*N*-ethyl-1,4-dihydropyridine.
(VI) 4-(4-nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-*N*-ethyl-1,4-dihydropyridine.

2.3. Electrolytic Media

All compounds were dissolved in 30 % ethanol and diluted with H_2O to obtain final concentrations varying between 0.05 – 5.0 mM. A routine concentration of 1 mM of 1,4-DHP derivatives was used. Solutions were bubbled with extra pure nitrogen (Indura, $H_2O < 3$ ppm, $O_2 < 2$ ppm $H_nH_m <$ 0.5 ppm) before each determination. Also, Britton-Robinson buffer was used, which consisted in acetic acid, phosphoric acid and borate acid in a final concentration of 0.04 M of each one.

2.4. Voltammetry

Cyclic voltammetry was performed with a BAS CV50 assembly. A glassy carbon was employed as working electrode. A platinum wire was used as a counter electrode and all potentials were measured against an Ag/AgCl electrode.

2.5. UV-Vis Spectroscopy

UV-vis spectra for each derivative were recorded in the 200 – 700 nm ranges at different intervals. The progress of CV or Controlled Potential Electrolysis (CPE) was followed using an UNICAM UV-3 spectrophotometer.

2.6. Spectroelectrochemical Cell

The cell contained a semitransparent platinum electrode formed by two parts: (a) two quartz plates adhered by Araldite and among them a platinum mesh is immersed (working electrode). (b) A bucket where both electrodes reference and auxiliary are located.

2.7. Controlled Potential Electrolysis (CPE)

CPE were carried out on a glassy carbon mesh electrode in 0.04 M Britton Robinson buffer / ethanol 70/30 at pH 7.4, with 0.1 M KCl. The potential applied varied between + 0.9, and + 1.05 V depending of 1,4-DHP. Oxygen was removed with pure and dry pre-saturated nitrogen. A three-electrode circuit with an Ag/AgCl electrode was used as reference and a platinum wire as a counter electrode. A BAS-CV 50 assembly was used to electrolyze the different derivatives.

2.8. HPLC Analysis on the Oxidation of 1,4-DHP

2.8.1. Equipment and Operation Conditions

HPLC measurement were carried out by using a Waters assembly equipped with a model 600 controller pump, model 464 pulsed electrochemical detector, and a model 996 photodiode array detector. The acquisition and treatment of data were made by means of the Millennium version 2.1 software. As chromatographic column a μBondapak/μPorasil C-18 column (3.9 × 150 mm) was used. As precolumn a C-18 μBondapak precolumn (30 × 4.6 mm) was employed. The injector was a 20 μL Rheodyne valve.

The working electrode in the electrochemical cell was a glassy carbon electrode and Ag/AgCl and Pt were used as reference and counter electrodes, respectively.

An aliquot of the electrolyzed solution of each 1,4-dihydropyridines was taken and a 20 μL volume of these solutions were injected into the chromatographic system. A mixture of Methanol/ 0.05 M phosphate buffer (55/45) at pH 4.3 was used as mobile phase.

The electrochemical (EC) detector was operated in the pulse mode, with 1400 mV as the oxidation applied potential. The photodiode array (PDA) detector operated at 250 nm for quantification. 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 below described, an injection volume of 20 μL was used.

2.8.2. Calibration Plots

Stock solution of 1 mM concentration in 0.04 M Britton-Robinson/ethanol (70/30) of each 1,4-DHP studied were used to prepare the respective calibration curves. Calibration drugs covering the range 0.01 – 0.2 mM were prepared from the stock solution in 0.04 M Britton-Robinson/ethanol (70/30). The calibration curves were obtained by plotting peak area versus the nominal concentration of each compound. The slope and intercept of the calibration lines were determined by unweighed least-squares.

2.8.3. Reproducibility

The reproducibility was determined from solutions of each 1,4-DHP with a final concentration of 0.08 mM. Reprodu-

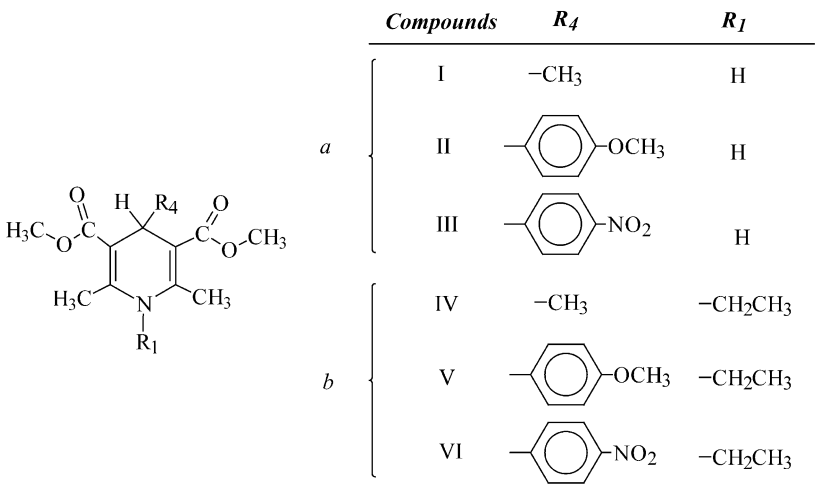

Fig. 1. Chemical structures of the 1,4-DHP compounds.

cibility was expressed as relative standard deviation (% R.S.D. = [standard deviation/mean of the measure] × 100).

2.8.4. Repeatability and Detection Limit

The intra-day and inter-day repeatability was determined by injecting replicate samples (n=10) of each 1,4-DHP and which were expressed as R.S.D. The detection limit of the method was defined as the quantity of 1,4-DHP required for a signal-to-noise ratio of 3.

3. Results and Discussion

The aim of this paper was to examine the electrochemical oxidation of a series of six non-commercial 1,4-dihydropyridine derivatives (Figure 1, I – VI), following the spectral changes by in situ spectroscopic measurements. Also, the HPLC technique was used to follow the time-course in the bulk electrolysis.

In previous studies [31, 32], we have found that the electro-oxidation of these compounds corresponded to a process that proceeds via 2-electrons, two protons. Cyclic voltammetry of these 1,4-DHP derivatives (Figure 1) on a glassy carbon electrode revealed an irreversible anodic signal at sweep rates varying between 0,1 V and 10 V, due to the oxidation of 1,4-dihydropyridine presumably to the corresponding pyridine derivative. However, the electrochemical generation of 1-electron oxidation intermediates in these compounds was previously confirmed by controlled potential electrolysis (CPE) and EPR experiments. By using *N-tert* butyl α-phenyl nitrone (PBN) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as the spin trap, these unstable radical intermediates were intercepted [32]. These results are consistent with previous works of other groups [7, 33]. As can be seen in Table 1, the order of oxidation easiness determined by DPV on glassy carbon electrode was: IV > V > I > II > VI > III. This result fairly agrees with the electron donating character of the substituents on the 4-position in the dihydropyridine ring.

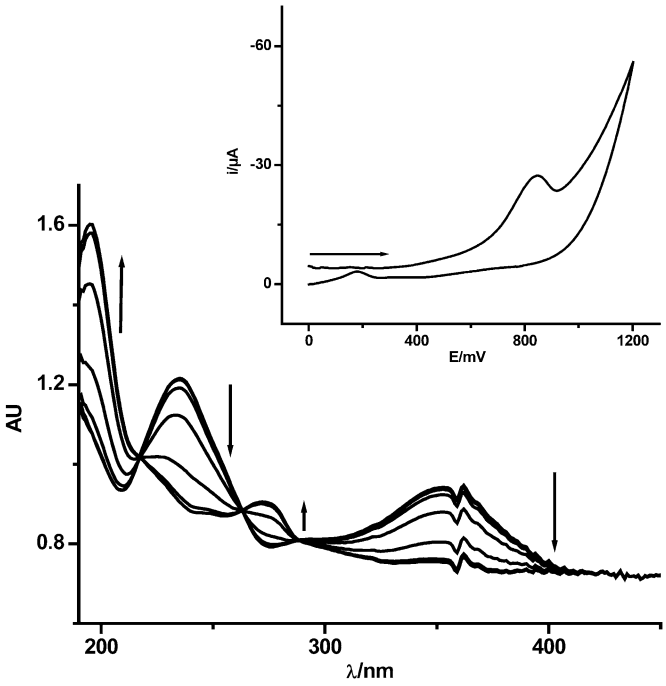

Fig. 2. Time-course of UV-vis spectra of 1 mM compound I solution in protic media (H_2O/EtOH 70/30 in 0.1 M $NaClO_4$) during a single cyclic voltammogram. Insert: single cyclic voltammogram.

3.1. Spectroelectrochemical Studies

All the 1,4-DHP derivatives exhibit two absorption bands at approximately 230 nm and 350 nm. As can be seen in Figure 2, when applying an anodic potential (CV) close to peak oxidation (Figure 2, insert) these bands diminish its intensity. In parallel with these changes, the appearance of a

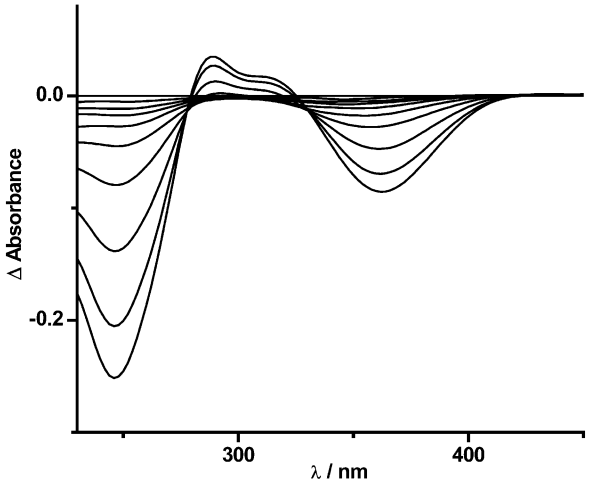

Fig. 3. Differential UV-Vis spectra of 1 mM compound V solution in protic media (H_2O/EtOH 70/30 in 0.1 M $NaClO_4$) during a single cyclic voltammogram.

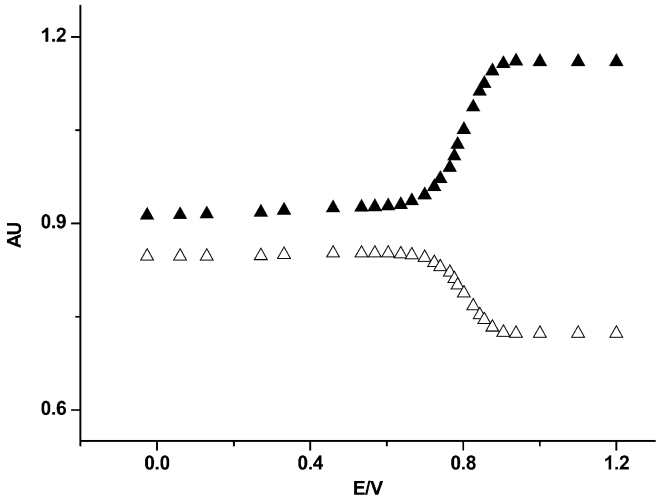

Fig. 4. Absorbance changes at 274 nm (▲) and 356 nm (△) versus applied potential of 1 mM compound II in H_2O/EtOH 70/30, 0.1 M $NaClO_4$.

new band at approximately 270 nm for the N-unsubstituted 1,4-DHP derivatives was noted. However, for the N-substituted 1,4-DHP derivatives a new UV band absorption at approximately 282 nm was formed (Table 1).

According to Labudzinska et al. [34] the UV band absorption near to 270 nm would correspond to the pyridine derivative formation. The shift in the UV absorption band to the 282 nm region for the N-substituted 1,4-DHP compounds could be due to the pyridinium salt formation [6]. Spectroelectrochemical studies of the resting 1,4-DHP exhibited similar results as shown in Table 1. Clearly from these results, during the cyclic voltammetry no unstable intermediates were detected.

In Figure 3, a typical differential UV spectrogram corresponding to the compound V (Figure 1) is displayed. As can be seen from this Figure, the original bands of this 1,4-DHP (246 nm; 363 nm) disappeared during the CV, concomitantly with the appearance of two new bands (288 nm; 314 nm). Results from these experiments for the resting of 1,4-DHP are summarized in Table 1.

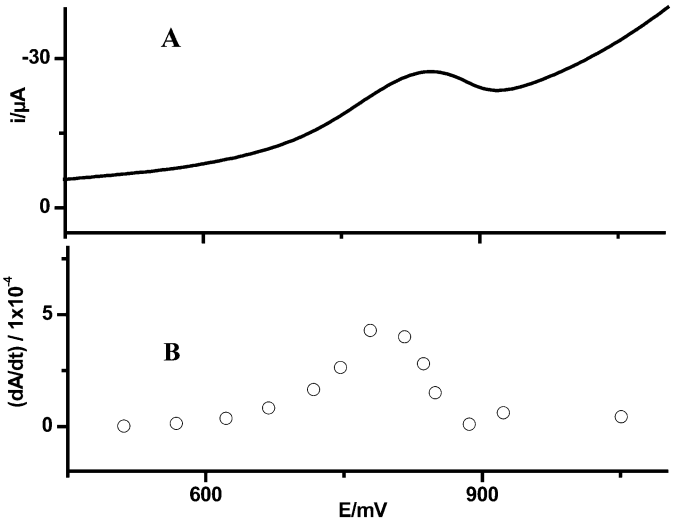

Fig. 5. VC (A) and DCVA (B) of 1 mM compound I in H_2O/Ethanol [70/30] with $NaClO_4$ 0.1 M. Scan rate 25 mV/min. DCVA was obtained from the spectrum of absorbance change at 275 nm with potential scan.

Table 1. Spectral and Chromatographic characteristics of parent 1,4-DHP and their corresponding oxidation products.

Compound	Peak potential [a] (mV)	λ_{max} [b] 1,4-DHP	λ_{max} [b] Pyridine	*Rt* (min) 1,4DHP [c]	*Rt* (min) Pyridine [d]
I	784	355	275	4.7	3.6
II	793	356	274 – 311,	6.0	7.3
III	858	288 – 372	270	8.7	7.7
IV	753	352	284	9.9	2.8
V	768	354	288 – 314	12.6	2.9
VI	820	290 – 346	282	16.0	2.8

[a] Average of oxidation peak potentials obtained by differential pulse voltammetry in aqueous media pH 4.0.
[b] Spectral characteristics obtained from spectroelectrochemical experiments.
[c] Average of retention times measured by both detectors (PDA & EC).
[d] Average of retention times measured by PDA detector ($\lambda = 250$ nm).

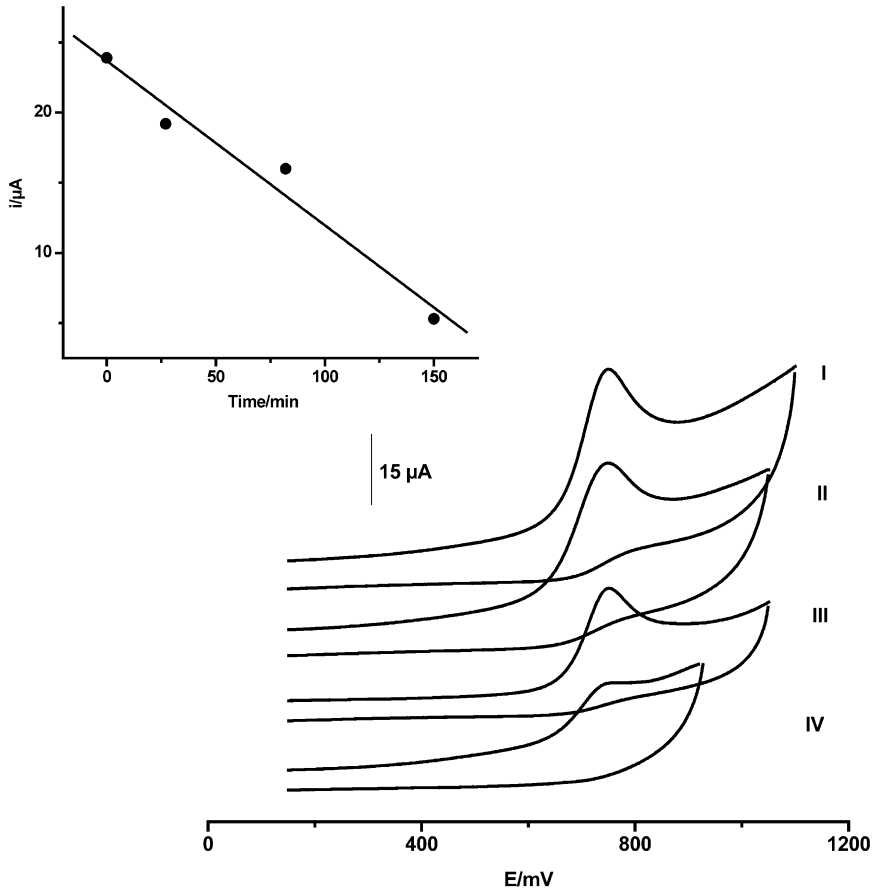

Fig. 6. Time-course of CPE of 1 mM compound I in aqueous media (Britton Robinson buffer 0.04 M/ethanol 70/30 at pH 7.4) following by cyclic voltammetry (0.5 V/s) on a glassy carbon electrode. I) 0 min, II) 27 min, III) 82 min, IV) 150 min. Insert: Time-course of peak current.

On the other hand, in the Figure 4 the typical absorbance changes in the UV absorption bands at 274 nm and 356 nm as a function of anodic applied potential for compound II is presented. It can be seen that at potentials lower than + 0.61 V, no changes were noted. However, from an anodic potential of + 0.61 V a significantly increase in the band at 274 nm parallel with a decrease in the band at 356 nm were observed. Oxidation potential values obtained from this type of plot are in accordance with those previously determined by cyclic voltammetric experiments.

From data relating absorbance changes with time for all the 1,4-DHP derivatives, we were able to get the so-called derivative cyclicvoltabsorptograms (DCVA). A typical DCVA for the compound I is shown in Figure 5. If non-faradaic components such as double layer charging are not involved in an ordinary cyclic voltammogram (CV), DCVA and CV should be identical in their shapes and normalized magnitudes in signals. As can be seen in Figure 5, both DCVA and CV shapes were similar as predicted when the electrode process is not complicated with superficial phenomena [35].

From the spectroelectrochemical experiments it can concluded that for all the studied 1,4-DHP derivatives no intermediates were detected and the spectral changes observed corresponded to the final oxidation products (pyridine or pyridinium salt derivatives). In general terms, the N-unsubstituted 1,4-DHP showed a new UV absorption band in the region of 270 nm during the CV, but the N-substituted 1,4-DHP presented new absorption bands in the region of 282 – 288 nm, which are consistent with the formation of different final oxidation product. In a previous study [21, 32], we had confirmed by GC/MS technique that N-unsubstituted 1,4-DHP formed the pyridine derivatives in the course of the oxidation process and as a final product. In the case, of the N-substituted 1,4-DHP applying the GC/MS technique no new fragments corresponding to final oxidation products were found [21].

3.2. Controlled Potential Electrolysis Studies

3.2.1. Cyclic Voltammetry

In Figure 6, the time-course of CPE corresponding to compound I is shown. It is observed, that the signal corresponding to the oxidation of the dihydropyridine ring diminished, but no new signals (intermediates or final products) in the range of potential studied were observed. In

addition, after 150 minutes of electrolysis practically all the original compound has been consumed. This technique is not adequate to follow the appearance of final oxidation products. Furthermore, the later reduction of the pyridine derivative could not be followed by the different tested working electrodes (Hg or glassy carbon electrodes).

3.2.2. HPLC Studies on the Oxidation of 1,4-DHP

For these studies the following mobile phase was selected from preliminary experiments: 0.05 M buffer phosphate/ Methanol (55/45) at pH 4.3. The relative standard deviation of the retention time was $<1\%$ for both detectors, thus indicating high stability of the system. On the other hand, linearity occurred at least from 0.01 mM to 0.2 mM for both detectors (PDA detector, $AUC = -163725.5 + 4.9527 \times 10^{10}$ (M), c.c. 0.99989; EC detector: $AUC = -5571 + 1.14088 \times 10^{10}$ (M), c.c. 0.9991). The average of repeatability was 1.2% and the detection limit was 2.1×10^{-6} M and 1.4×10^{-6} M for PDA and EC detectors, respectively. The average reproducibility was 1.8%.

a) HPLC Characterization of 1,4-DHP derivatives with the PDA detector

Retention times determined for both parent 1,4-DHP (Figure 7A) and their corresponding oxidation products are summarized in Table 1. As can be seen, retention time values for the pyridine derivatives were closer than those parent 1,4-DHP derivatives. In contrast, retention time values for the pyridinium salt derivatives became to be significantly lower than their corresponding parent N-substituted 1,4-DHP compounds. In addition, it was observed that the retention times of the oxidized derivatives corresponding to compound I and III, were smaller than the parent derivatives. On the other hand, for compound II, its oxidized derivative presented greater retention time than the respective parent compound.

In Figure 8, the time-course of CPE followed with PDA detector for compound II 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 9 minutes of electrolysis, a second signal corresponding to an oxidation product was evidenced. In the insert of Figure 8, the evolution of the area under the curve (AUC) corresponding to both, the parent 1,4-DHP and its pyridine derivative is shown. A similar behavior was found for the resting compounds.

In conclusion, the main advantage of this detector resides in the simultaneous determination of both the parent 1,4-DHP derivative and its oxidation product generated in the course of the bulk electrolysis.

b) HPLC Characterization of 1,4-DHP derivatives with the EC detector

For these chromatographic measurements a glassy carbon working electrode in the oxidation pulse mode was used. The main chromatographic characteristics obtained with this detector are summarized in Table 1. As can be seen, the N-substituted 1,4-DHP exhibited retention times longer than that of the corresponding parent drugs. The decrease in polarity as a consequence of the *N*-alkylation produced an increase close to 2-fold in the retention times in these derivatives compared with the N-unsubstituted ones. In any case, both the compound I and IV bearing a methyl group in 4-position presented the smallest retention time. All the compounds exhibited only one signal in this mode (Figure 7B). The rank order of the retention time is as follows: $I < II < III < IV < V < VI$. In conclusion, HPLC with EC detector did not permit the determination of electrolysis products.

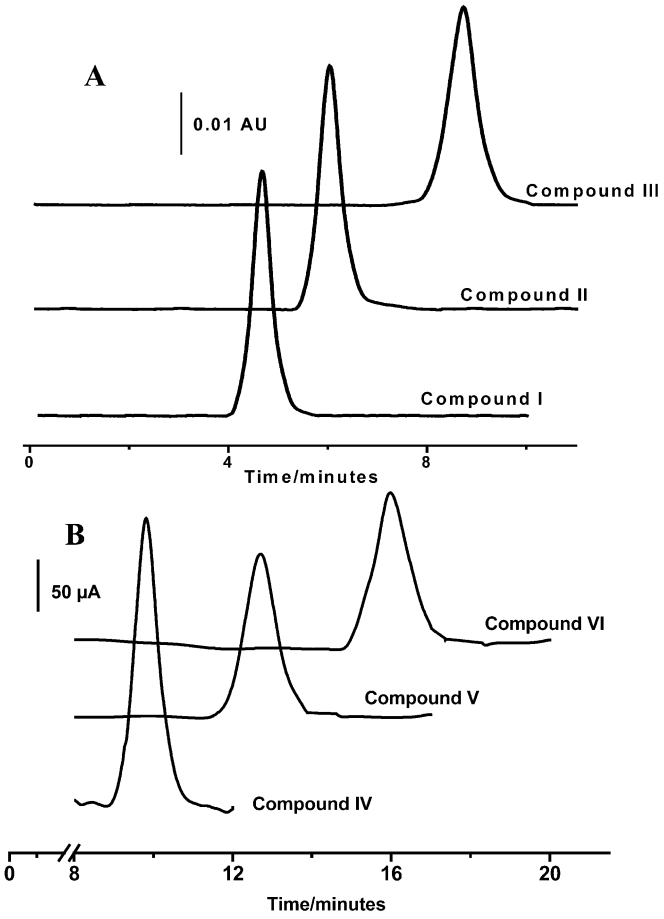

Fig. 7. HPLC chromatograms. A) signals obtained with PDA detector of parent 1,4-DHP derivatives and B) signals obtained with EC detector of *N*-ethyl derivatives in 50 mM phosphate buffer/Methanol [45/55] at pH 4.3. Flux: 1 mL/min at 35 °C.

4. Conclusions

From the spectroelectrochemical studies it can be concluded that was possible to follow the appearance of final products of oxidation corresponding to the 1,4-DHP series, which presented different spectral characteristics depending of the nature of the N-substitution. Thus, N-unsubstituted 1,4-DHP exhibited absorption bands close to the region of 270–275 nm and 310 nm. However, the absorption bands corre-

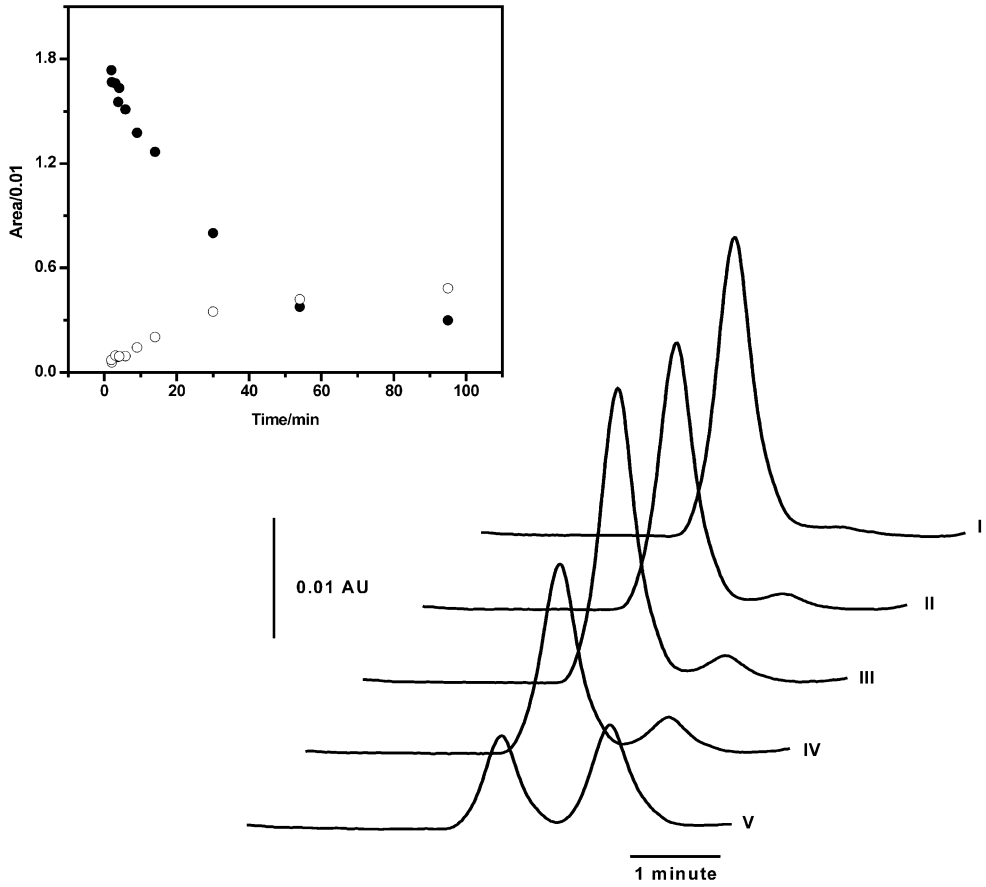

Fig. 8. Time-course of CPE of compound II (100 μM) in aqueous media followed by HPLC with PDA detector. I) 3 minutes, II) 9 minutes, III) 16 minutes, IV) 30 minutes, V) 54 minutes. Insert: Evolution of area under curve of CPE in aqueous media. (●) peak at 6.0 minutes (compound II) and (○) peak at 7.3 minutes (pyridine).

sponding to the *N*-ethyl substituted 1,4-DHP were shifted to the region of 282 – 288 nm and 314 nm.

Results provided by HPLC with the PDA detector demonstrated that in the selected conditions, the method was adequate to follow the changes occurring in the oxidation process of 1,4-DHP (CPE experiments), permitting the simultaneous determination of the concentration of both parent 1,4-DHP and their oxidation products (pyridine or pyridinium salt).

5. Acknowledgements

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