# Oxidation of 4-(3-Indolyl)- and 4-(5-Indolyl)-1,4-dihydropyridines in Aprotic and Protic Media: Reactivity toward Alkylperoxyl Radicals

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Electrochemical oxidation of 4-(3-indolyl)- and 4-(5-indolyl)-1,4-dihydropyridines (DHPs) in aprotic and protic media is reported. Also, the reactivity of compounds toward alkylperoxyl radicals 2,2'-azobis-(2-amidinopropane) dihydrochloride-derived in aqueous media at pH 7.4 is assessed. Derivatives were electrochemically oxidized exhibiting two anodic signals in both electrolytic media. The first signal is due to oxidation of the dihydropyridine ring, and the second one is due to oxidation of the indolyl moiety. Electron spin resonance experiments proved the formation of carbon-centered dihydropyridyl radicals as intermediates in the oxidation of the dihydropyridine moiety in aprotic medium. Pyridine was identified as the final product of the oxidation in both electrolytic media by gas chromatography/mass spectrometry. The 4-substituted 1,4-DHPs were more reactive than tested commercial 1,4-DHPs toward alkylperoxyl radicals.

The 4-substituted Hantzsch dihydropyridines, analogs of NADH coenzymes, are an important class of drugs. The 1,4-dihydropyridines (DHPs) are allosteric modulators that act on L-type $Ca^{2+}$ channels in a voltage-dependent manner either as antagonists or agonists.[1] The former is clinically used for the treatment of cardiovascular diseases, including hypertension.[2,3] In the human body, these compounds undergo oxidation to form pyridine derivatives. These oxidized compounds are largely devoid of the pharmacological activity, when compared to their parent compounds.

To reach more selective and long-acting drugs with fewer side effects, structural modifications have been made. Changes in the substituent pattern of the C-3, C-4, and C-5 positions of the dihydropyridine ring alter activity and tissue selectivity.[4,5]

Our laboratory has been working on the synthesis and electrochemical characterization of DHPs for several years. This effort has focused on the synthesis of derivatives with stronger antioxidant properties and the study of the interactions of different redox centers coexisting in the same molecule. We considered the indole as a good choice for a second redox center because it has been reported to possess a wide variety of biological properties, including anti-inflammatory, antibacterial, anticonvulsant, and antioxidant properties.[6-8] Consistent with the above-mentioned facts, in this article the synthesis and electrochemical characterization of two compounds [4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP], both having a dihydropyridine ring and an indole ring acting as redox centers in the same molecule, are studied. Lavilla et al.[9] have previously reported the design, synthesis, and pharmacological evaluation of a series of 4-(3-indolyl)dihydropyridines. The tested DHPs showed the same potency as nifedipine but lower efficacy in blocking the KCl-contractions in rat aorta and vas deferens (part of the male reproductive system in humans). The inhibited production of radical oxygen-derived species was also similar to that of nifedipine, being related to the antioxidant properties of dihydropyridines, regardless of their substitution pattern.

From the electrochemical point of view, the study of the oxidation of some 1,4-DHP has previously been performed preferentially in nonaqueous media using mainly a rotating ring-disk electrode, linear and cyclic voltammetry (CV), and controlled-potential electrolysis coupled with electron spin resonance (ESR) spectroscopy.[10-14] In this framework, the electrochemical oxidation of a series of new DHPs in aprotic and protic media has been also reported by our group.[15-19]

In addition, in this paper the electrochemical oxidation in both aprotic and protic media of two synthesized and characterized 4-indolyl 1,4-dihydropyridines is reported. Results are compared to the 4-phenyl substituted 1,4-dihydropyridine derivative. The reactivity of compounds toward alkylperoxyl radicals 2,2'-azobis-(2-amidinopropane) dihydrochloride (ABAP)-derived at pH 7.4 is also assessed.

## Experimental

*C4-indolyl substituted 1,4-dihydropyridines (Fig. 1).*— A mixture of 6 mmol of indole 3-carboxaldehyde or indole 5-carboxyaldehyde in 20 mL of ethyl alcohol was mixed with 0.015 mol of ethyl acetoacetate and 0.01 mol of concentrated ammonium hydroxide. This mixture was heated under reflux for 30 h

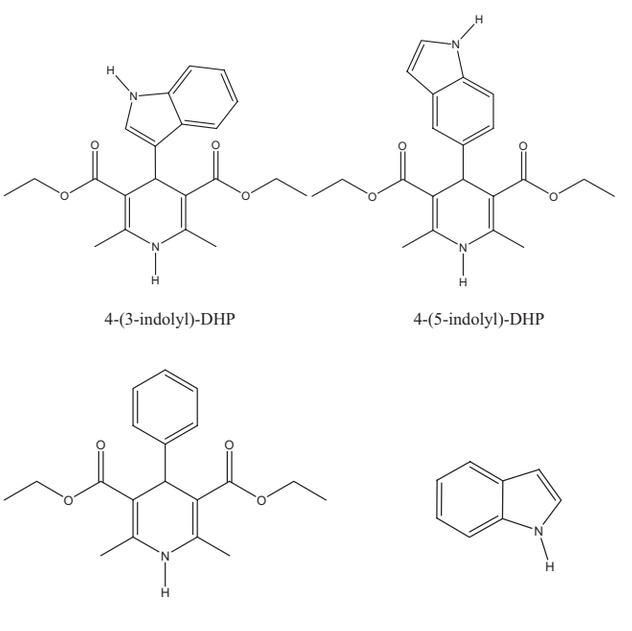


**Figure 1.** Chemical structures of 4-(3-indolyl)-DHP, 4-(5-Indolyl)-1,4-DHP, 4-phenyl-DHP, and indole.

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under nitrogen. The crude solid obtained was filtered and recrystallized from ethyl alcohol/water (50/50). Synthesized compounds had the following characteristics.

*2, 6-dimethyl-3, 5-diethoxycarbonyl-4-(3-indolyl)-1, 4-dihydropyridine [4-(3-indolyl)-DHP].*— Yield: 73%. mp.:183–184°C. IR (KBr): 3344.4; 2978.1; 1676.7; 1487.1; 1367.9; 1305.6; 1215.4; 1100.3; 1020.4; 807.2; 744.7 $cm^{-1}$. $^1$H-NMR (300 MHz, dimethyl sulfoxide (DMSO)-$d_6$): $\delta_{max}$ 1.18 (t, 6H, 2x $-CH_2CH_3$); 2.31 (s, 6H, 2x $-CH_3$); 4.03 (q, 4H, 2x $-OCH_2CH_3$); 5.23 (s, 1H, ArCH); 6.97 (m, 3H, $J$ = 7.83 Hz, 3x ArH); 7.33 (d, 2H, $J$ = 7.92 Hz, 2x ArH); 7.92 (d, 2H, $J$ = 7.92 Hz, 2x ArH); 8.89 (s, 1H, DHP-NH); 10.74 (s, 1H, indole-NH). $^{13}$CNMR (75 MHz, DMSO-$d_6$): 13.70; 17.64: 29.98; 58.25; 101.43; 110.85; 117.54; 118.91; 119.77; 121.31; 122.17; 125.25; 135.54; 143.58; 166.71. Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C 68.46; H 6.57; N 7.60. Found: C 68.16; H 6.55; N 7.61.

*2, 6-dimethyl-3, 5-diethoxycarbonyl-4-(5-indolyl)-1, 4-dihydropyridine [4-(5-indolyl)-DHP].*— Yield: 61%. mp.:178–180°C. IR (KBr): 3351.2; 2978.5; 1657.6; 1481.6; 1371.4; 1303.5; 1208.2; 1100.5; 1018.2; 727.0 $cm^{-1}$. $^1$HNMR (300 MHz, DMSO-$d_6$): δ 1.20 (t, 6H, 2x $-CH_2CH_3$); 2.32 (s, 6H, 2x $-CH_3$); 4.04 (q, 4H, 2x $-OCH_2CH_3$); 4.99 (s, 1H, ArCH); 6.37 (s, 1H, ArH); 7.00 (d, 1H, J = 7.92 Hz, 1x ArH); 7.25 (m, 3H, J = 7.56 Hz, 3x ArH); 8.76 (s, 1H, DHP-NH); 10.94 (s, 1H, indole-NH). $^{13}$CNMR (75 MHz, DMSO-$d_6$): 13.74; 17.96: 48.802; 58.37; 100.20; 102.53; 110.01; 117.72; 120.76; 124.49; 127.07; 134.08; 138.52; 143.88; 166.71. Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C 68.46; H 6.57; N 7.60. Found: C 68.70; H 6.60; N 7.58.

*2,6-dimethyl-3,5-diethoxycarbonyl-4-phenyl-1,4-dihydropyridine (4-phenyl-DHP) was synthesized according to a previous paper 6.*— Yield: 92%. mp.:150–153°C. $^1$HNMR (300 MHz, DMSO-$d_6$): δ (1.16 (t, 6H, 2x $-CH_2CH_3$); 2.26 (s, 6H, 2x $-CH_3$); 3.98 (q, 4H, 2x $-OCH_2CH_3$); 4.88 (s, 1H, ArCH); 7.18 (m, 3H, $J$ = 6.975 Hz, 3x ArH); 7.22 (d, 2H, $J$ = 8, 14 Hz, 2x ArH); 8.80 (s, 1H, NH). $^{13}$CNMR (75 MHz, DMSO-$d_6$): 10.51; 9.26; 9.07; 7.88; 7.78; 6.18; 2.09; 2.05; 2.04; 1.97; 0.63. Anal. Calcd. for $C_{19}H_{23}O_4N$: C:62.28; H: 7.04; N: 4.25. Found: C: 62.08; H: 6.98; N: 4.30.

*Electrolytic media.*— Aprotic medium: Dimethyl sulfoxide (DMSO) containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF6). Protic medium consisted of 0.04 M Britton–Robinson buffer/ethanol: 70/30 and adjusted to an ionic strength of 0.1 M with KCl.

*Voltammetry.*— Differential pulse (DPV) and CV were performed with a bioanalytical system (BAS)-CV 100 assembly. All voltammetric experiments were carried out with 1 mM solutions of DHPs. A stationary glassy carbon electrode was used as the working electrode (0.07 $cm^2$) for DPV and CV experiments. For hydrodynamic experiments, a rotating disk glassy carbon electrode (0.07 $cm^2$) was employed. The surface of the electrode was polished to a mirror finish with alumina powder (0.3 and 0.05 μm) before use and after each measurement. Platinum wire was used as an auxiliary electrode, and all potentials were measured against an Ag/AgCl electrode in aqueous saturated KCl.

*Controlled-potential electrolysis (CPE).*— Exhaustive electrolysis at a reticulated glassy carbon electrode was carried out at the following potentials: Aprotic medium: 1100 and 1500 mV. Protic medium: 900 and 1300 mV at pH 3. At pH 11, the applied potentials were 550 and 700 mV. A BAS-CV 100 assembly was used to electrolyze the 1,4-DHPs solutions. Net charge was calculated, including correction for the background current.

*UV/vis spectrometry.*— UV/visible (UV/vis) spectra were recorded in the 200–1000 nm range by using an Agilent spectrophotometer with a diode array using 1 cm quartz cell.

*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. The hyperfine splitting constants were estimated to be accurate within 0.05 G. Electrolysis was performed in the ESR cell using an appropriate platinum mesh electrode according to the same conditions as described above by using 1 mM 1,4-DHP solutions in the presence of 100 mM N-*tert*-butylamine-α-phenylnitrone (PBN), which was used as spin trap.

*GC-MS.*— A gas chromatography/mass spectrometry (GC-MS) detector (5890/5972) combination (Hewlett-Packard, Palo Alto, CA, USA) and a Hewlett-Packard 7673 autosampler were used for the analyses. The $m/z$ range monitored was 45–550 with a scan rate of 1 scan/s; the normal electron energy was 70 eV. A Hewlett-Packard Ultra-1 column, 25 cm–0.2 mm id–0.11 mm film thickness (Little Falls, Wilmington, DE, USA), was used.

*Reactivity toward alkylperoxyl ABAP-derived radicals.*— ABAP [2,2'-azobis-(2-amidinopropane) dihydrochloride]. Different series of 100 mM ABAP solutions in 0.04 M Britton–Robinson buffer/*N*,*N*-dimethylformamide (DMF) 70/30 pH 7.4 at a constant ionic strength of 0.1 M adjusted with KCl were incubated with different solutions (20–200 μM) of 1,4-DHP synthesized and commercial 1,4-DHPs at 37°C for 120 min with constant bubbling of oxygen. From these experiments, reaction rates were calculated for each 1,4-DHP derivative. The rate of alkylperoxyl radical formation from ABAP is constant at a given temperature.[20,21] However, the rate of formation of alkylperoxyl radicals from this initiator will not be constant because it depends on the concentration of ABAP (rate = $k$[ABAP]). It appears that over 120 min at 37°C, only a small amount of the ABAP will decay; therefore, the rate may be considered constant at 37°C. In neutral aqueous solutions, the half-life of ABAP is about ~175 h, and the generation rate of radicals is constant for the first few hours.[20,21] Control solutions containing either DHP solution were run under the same conditions as the above mixtures. The time course of the reactivity of synthesized 1,4-DHP derivatives with the generated alkylperoxyl radicals was followed by UV/vis spectroscopy and GC-MS techniques.

According to the previous text, we can assume that the high [ABAP] decomposes slowly at 37°C, but it maintains [$ROO^{\cdot}$] at least 25-fold greater than [DHP]'s to ensure pseudo first-order conditions.

As can be seen later, in Fig. 7, plots of ln [DHP] vs time are linear with a slope value equal to $k'$. The linearity of the plots [$r$ = 0.9997 for 4-(3-indolyl)-DHP and $r$ = 0.9994 for 4-(5-indolyl)-DHP] supports the original assumption of pseudo first-order kinetics for the reaction. Rate constants for 1,4-DHP were calculated from five independent experiments. Also the reactivity toward alkylperoxyl radicals was compared to commercial 1,4-DHPs by using the following ratio ($r$)

$$r = k \text{ value commercial 1,4-DHP}/k \text{ value DHP tested}$$

Control solutions (in the absence of ABAP-derived radicals) revealed no changes either in their original UV/vis absorption bands or GC-MS fragmentation. Also, a possible photodecomposition of 1,4-dihydropyridines was assessed, but in the time scale of the experiments this was negligible.

## Results

*Aprotic medium.*— The main goal of the present work was the study of the oxidation of two synthesized 4-indolyl-1,4-dihydropyridines to investigate the possible reciprocal interactions between two redox centers coexisting in the molecule (i.e., the 1,4-dihydropyridine ring and the indole moiety).

In Fig. 2A, differential pulse (DP) voltammograms on a glassy carbon electrode for the different derivatives are shown. As can be seen, 4-indolyl-DHP derivatives exhibits two well-defined oxidation signals. A first oxidation signal was found between 944 and 992 mV, for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively.

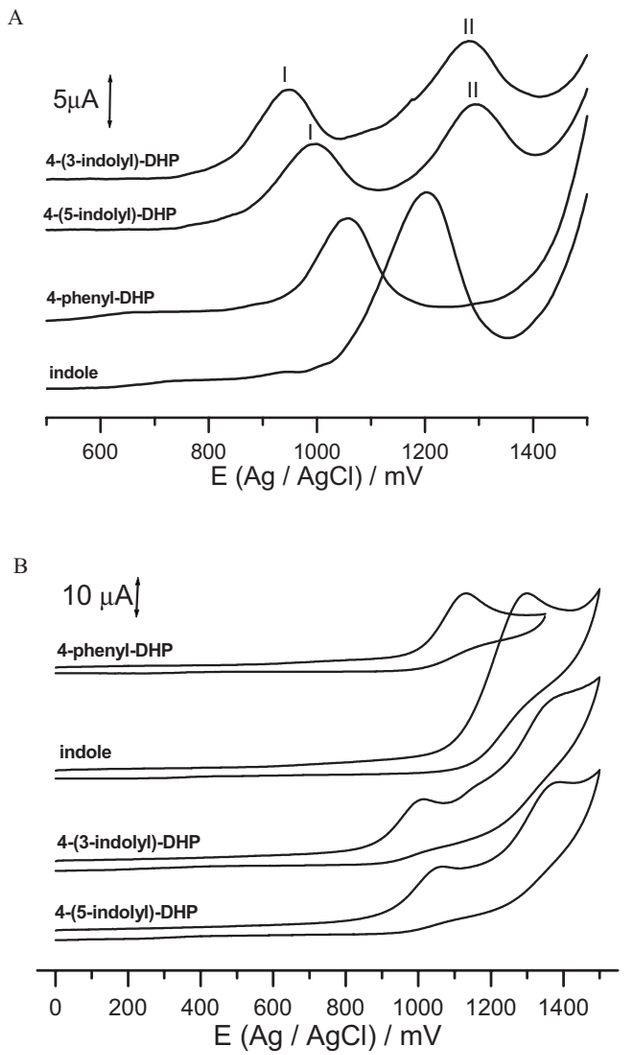


**Figure 2.** DPV (A) and cyclic voltammograms at 0.1 V/s (B) of 1 mM solutions of 4-(3-indolyl)-DHP, 4-(5-indolyl)-DHP, 4-phenyl-DHP, and indole in dimethylsulfoxide +0.1 M TBAPF6.

These signals can be assigned to the oxidation of the dihydropyridine ring by comparison to the oxidation potential of the reference compound (4-phenyl-DHP in Fig. 2A). A second oxidation signal appears at peak potentials between 1288 and 1292 mV, for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively. Likewise, we have concluded that these oxidation peaks correspond to the indole moiety by comparison to the reference compound (indole in Fig. 2A). Complete data on oxidation peak potentials are summarized in Table I. As can be seen, oxidation potentials corresponding to the indole moiety are moved toward more positive values for both synthesized derivatives compared to the indole molecule. From an analysis of the behavior of 1,4-DHP moiety, it can be concluded that its oxidation is easier than that of 4-phenyl substituted 1,4-DHP.

Cyclic voltammograms corresponding to synthesized compounds are shown in Fig. 2B. Results are similar to those previously obtained by DPV. Both derivatives are irreversibly oxidized at all sweep rates studied (0.1–3 V $s^{-1}$). Plots of log $i$ vs log $v$ have slopes between 0.5 and 1.0, indicating that the current is controlled by a mixed process (diffusion and adsorption).

Typical voltammograms for a graphite rotating disk electrode corresponding to a 1 mM solution of 4-(3-indolyl)-DHP in aprotic medium are shown in Fig. 3. Both 4-indolyl substituted 1,4-DHP

**Table I. Oxidation peak potential values on glassy carbon electrode in aprotic (DMSO + 0.1 M TBAPF6) and protic (0.04 M Britton–Robinson buffer/ethanol (70/30) at pH 3 and pH 11) media.**

| Aprotic medium | E/mV (Ag/AgCl) | | | |
|---|---|---|---|---|
| Derivative | Peak I | ΔEp-DHP[a] | Peak II | ΔE p-Indole[b] |
| 4-(3-indolyl)-DHP | 944 | −120 | 1280 | +80 |
| 4-(5-indolyl)-DHP | 992 | −72 | 1288 | +88 |
| 4-phenyl-DHP | 1064 | — | — | — |
| Indole | — | — | 1200 | — |
| **Protic medium pH 3** | Peak I | ΔEp-DHP[a] | Peak II | ΔEp – Indole |
| 4-(3-indolyl)-DHP | 754 | −126 | 1112 | +162 |
| 4-(5-indolyl)-DHP | 774 | −106 | 1088 | +138 |
| 4-phenyl-DHP | 880 | — | — | — |
| Indole | — | — | 950 | — |
| **Protic medium pH 11** | Peak I | ΔEp-DHP[a] | Peak II | ΔEp-Indole[b] |
| 4-(3-indolyl)-DHP | 398 | −42 | 606 | +65 |
| 4-(5-indolyl)-DHP | 425 | −15 | 628 | +87 |
| 4-phenyl-DHP | 440 | — | — | — |
| Indole | — | — | 541 | — |

[a] Differences of oxidation peak potential values of 1,4-DHP ring with respect to 4-phenyl-DHP derivative.
[b] Differences of oxidation peak potential values of 1,4-dihydropyridine ring with respect to indole.

derivatives exhibit two oxidation waves (I-II). However, 4-phenyl-DHP exhibits only a single oxidation wave, which corresponds to oxidation of the dihydropyridine ring. In consequence, wave I is due to the oxidation of the dihydropyridine ring and wave II to the oxidation of the indole moiety. As can be seen, at low rotation rates (100–500 rpm), a little adsorption of the electroactive species is observed, which disappears at rotation rates of >800 rpm (Fig. 3). Similar behavior was found for the 4-(5-indolyl)-DHP derivative.

From Levich plots, the following diffusion coefficients were calculated: 4-(3-indolyl)-DHP = $2.5 \pm 0.2 \times 10^{-6}$ $cm^2$ $s^{-1}$ and 4-(5-indolyl)-DHP = $2.3 \pm 0.1 \times 10^{-6}$ $cm^2$ $s^{-1}$. As can be seen, no significant difference was found between the values for these two derivatives.

To understand the mechanism of oxidation in greater detail and to determine the number of electrons transferred in the oxidation

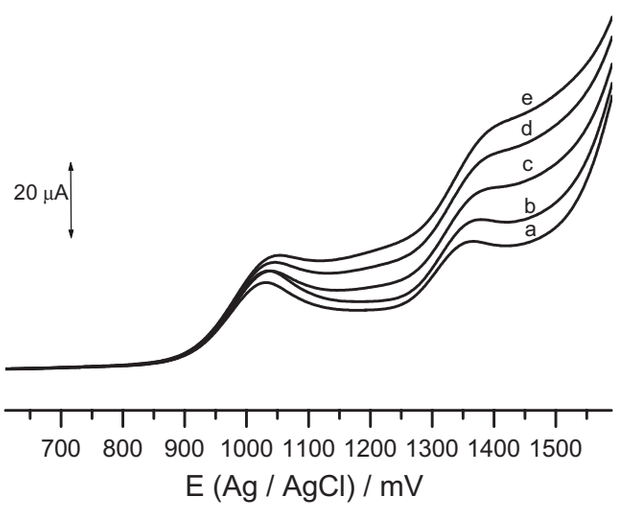


**Figure 3.** Typical hydrodynamic voltammograms on glassy carbon rotating disk electrode of 1 mM 4-(3-indolyl)-DHP in DMSO + 0.1 M TBAPF6 at different rotation rates: a = 100 rpm, b = 300 rpm, c = 500 rpm, d = 600 rpm, and e = 800 rpm.

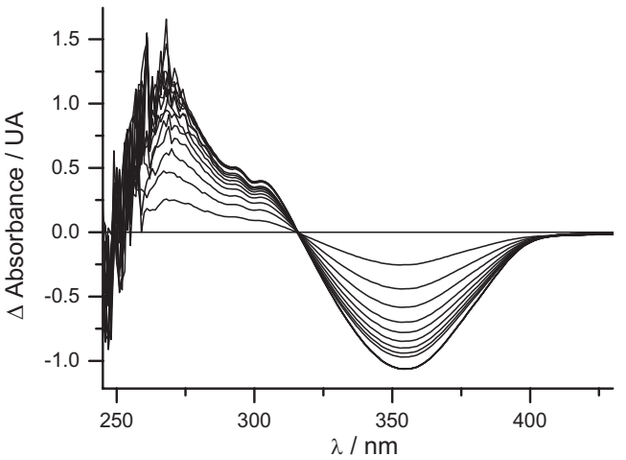


**Figure 4.** UV/vis differential spectra for the time course of CPE at +1100 mV for a 100 μM 4-(3-indolyl)-DHP solution followed during 90 min.

process, CPE studies were carried out in aprotic media. Solutions containing accurately weighed amounts of the C4-indolyl-DHPs with 0.1 M TBAPF6 were subjected to electrolysis at constant potential. For the first applied potential of 1100 mV, results from these experiments revealed an average $n$ value of 2.10 ± 0.20 for the DHPs. These values indicate that oxidation mechanism is a two-electron process oxidation of the 1,4-dihydropyridine ring. Solutions were electrolyzed at a potential of 1500 mV. Results from these experiments revealed an overall $n$ value of 3.60 ± 0.40. This value could correspond the oxidations of the 1,4-dihydropyridine ring and the indole moiety, involving two electrons and one electron, respectively. This latter value is consistent with previous reports in literature for the oxidation of indole.[22,23] The formation of a blue film on the reticulated carbon electrode was observed after 2 h of electrolysis at 1500 mV. These facts could explain an $n$ value higher than one electron.

Electrolyzed solutions were analyzed by UV/vis, ESR, and GC-MS techniques to identify possible intermediates and final oxidation product(s). Spectroelectrochemical experiments were conducted to determine either unstable intermediates or final products during the in situ controlled-potential electrolysis. The experiments were followed by changes on the UV/vis spectra during 90 min. The application of an oxidation potential of 1100 mV, close to the first anodic peak, produced a noticeable change in the spectrum of DHP derivatives as shown in Fig. 4. The UV/vis differential spectra for both derivatives reveals that, parallel with the decrease of the original UV absorption bands at 353 nm, a signal appears between 270 and 290 nm. These UV bands correspond to the oxidized derivative (i.e., the pyridine derivative, which is in agreement with previous observations).[16,18,24,25]

By applying an anodic potential close to the second oxidation peak (i.e., 1500 mV), no changes in the spectra were registered. From these results, it can be concluded that during controlled-potential electrolysis (CPE) no unstable intermediates are detected by this technique, and the signals correspond to the formation of pyridine derivative.

To identify the intermediates produced in the time course of the electrolysis of DHP derivatives, spin-trapping studies with PBN were conducted. At an applied potential of 1100 mV, the corresponding ESR spectra show a triplet, due to the nitrogen, and their splitting into a doublet is due to the presence of the adjacent hydrogen (Fig. 5A-C). The corresponding $a_N$ values were 13.9 and 14.0 G for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively. $a_H$ splitting values for the same compounds were 2.1 and 2.2 G, respectively. The above-splitting constants for the spin adducts are consistent with the fact that PBN interacts with carbon-centered radicals as reported for 1,4-DHP derivatives and for other structurally related compounds.[26] CPE at 1500 mV revealed a mixture of radical species for 4-(5-indolyl)-DHP as displayed in Fig. 5C. As can be seen, signals at 3.4174 and 3.451.7 G appeared, indicating the formation of other intermediates probably involving the indole moiety. However, the resolution of these types of spectra was not possible. ESR spectra of 4-(3-indolyl)-DHP did not show any change, possibly because the formation of indolyl radical is sterically hindered. In Fig. 5D, the spectrum of PBN in the absence of 1,4-DHP derivatives at an applied potential of 1500 mV is shown. As can be seen, at this oxidation potential PBN did not suffer any change.

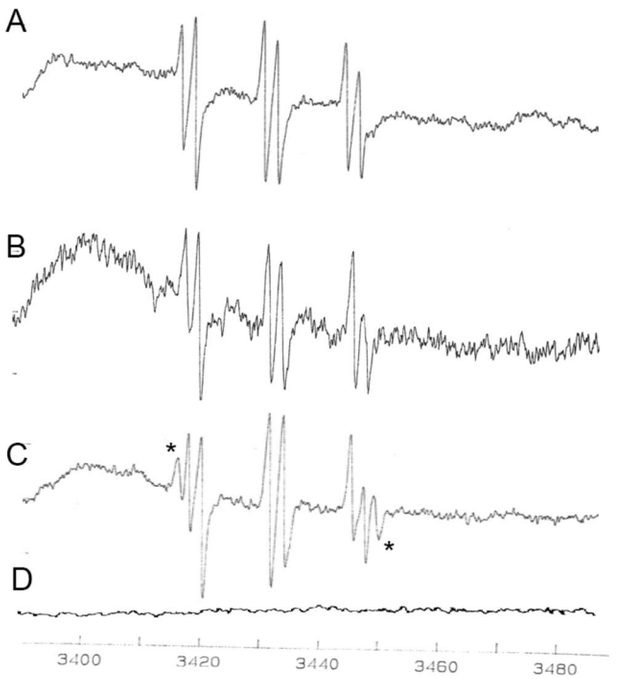


**Figure 5.** ESR spectra of 4-(3-indolyl)-DHP (A) and 4-(5-indolyl)-DHP (B) electrolyzed solutions at 1100 mV. (C) ESR spectrum of 4-(5-indolyl)-DHP electrolyzed at 1500 mV (* New signals). (D) ESR spectrum of 100 mM PBN electrolyzed at +1500 mV in DMSO +0.1 M TBAPF6.

*Protic media.*— To study the pH dependence of the oxidation process, we also tried voltammetry in aqueous buffers. In a similar way as for an aprotic medium, 4-indolyl-substituted 1,4-DHP derivatives exhibited two well-defined oxidation peaks (I and II) throughout the pH range (Fig. 6). Furthermore, in the inset, the $E_p$ vs pH behavior for 4-(3-indolyl)-DHP is shown. Peak I at $2 < \text{pH} < 4$ is pH independent, but at $\text{pH} > 4$ is pH dependent. In contrast, peak II was pH dependent in all the pH ranges (pH 2–12). These results imply that peak I, in both compounds, corresponds to oxidation by two different mechanisms. In the pH-independent zone, no protons are involved before or in the rate-determining step but in the pH-dependent zone protons are involved before or in the rate-determining step. In the case of peak II, the oxidation follows the same mechanism throughout the pH range. A similar behavior was observed for 4-(5-indolyl)-DHP. 4-phenyl-DHP and indole derivatives display a single peak related to oxidation of the 1,4-dihydropyridine ring and indole moiety, respectively. The electro-oxidation process of 4-phenyl-DHP derivative is similar to the oxidation represented by peak I (i.e., at $2 < \text{pH} < 4$, the process is pH-independent, and at $\text{pH} > 4$ is pH dependent). Indole presents an oxidation peak that is pH dependent throughout the pH range, like peak II.

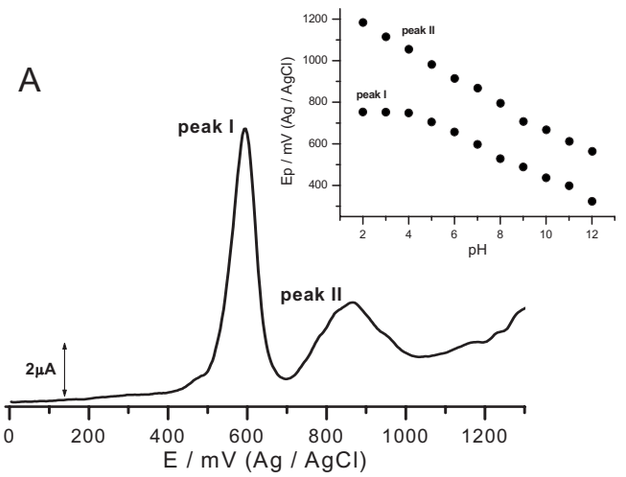


**Figure 6.** DP voltammogram of 1 mM 4-(3-indolyl)-DHP in 0.04 M Britton–Robinson buffer/ethanol (70/30) at pH 7. Insert: Ep/pH plot for 4-(3-indolyl)-DHP.

In a way similar to that for an aprotic medium, the first peak is assigned to oxidation of the dihydropyridine ring and peak II corresponds to oxidation of the indole moiety. As can be seen, the inclusion of the indole in the 4-position of the dihydropyridine ring led to the second oxidation process, related to the oxidation with the 4-phenyl-DHP derivative. However, the oxidation potentials corresponding to the indole moiety are shifted toward more positive values in both synthesized derivatives as compared to the indole molecule. The number of electrons transferred in the oxidation of the derivatives in protic media was determined by CPE at pH 3 and 11. Solutions containing an accurately weighed amount of the 4-indolyl-substituted 1,4-DHP derivatives in 0.04 M Britton–Robinson buffer were electrolyzed at a constant potential. At pH 3 and a potential of 900 mV, the average $n$ value was $2.16 \pm 0.20$ for the 1,4 DHPs. This value is in accord with a two-electron mechanism for the oxidation of the 1,4-dihydropyridine ring. Nevertheless, solutions were also electrolyzed at 1300 mV to give an $n$ value of $3.44 \pm 0.30$. This value corresponds to oxidation of the 1,4-dihydropyridine ring (two electrons, two protons) and the indole moiety involving one electron and one proton. At pH 11, a total average value of $2.5 \pm 0.20$ was found for electrolyzed solutions at 700 mV.

To identify the products of the oxidation reaction, GC-MS experiments were conducted. Synthesized DHPs follow different fragmentation pathways. In the case of 4-(3-indolyl)-DHP, the most abundant fragment had $m/z$ 295, which corresponds to complete loss of the lateral chain at the three positions. However,4-(5-indolyl)-DHP exhibited a different pathway. Thus, the most abundant fragment gave $m/z$ of 252, which is formed from the molecular ion by the complete loss of the indole moiety.[27] This change can be explained by the attachment of the indole moiety through the five positions of the indole moiety. Retention times ($R_t$) of the parent derivatives were 11.9 and 12.1 min for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively.

1,4-DHP solutions electrolyzed at 1100 mV for 90 min gave species with retention times of 10.4 and 10.8 min for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively. The most abundant signals obtained for electrolyzed solutions of 4-(3-indolyl)-DHP containing the pyridine nucleus were $M_+$ 366, $m/z$ 321, $m/z$ 291, and $m/z$ 247. In contrast, electrolyzed solutions of 4-(5-indolyl)-DHP derivative gave $M_+$ 366, $m/z$ 275, and $m/z$ 248 as the most abundant signals.

From these results, it can be concluded that the most abundant signal appears at $m/z$ 366, which means that the pyridine derivative is generated by electrochemical oxidation at 1100 mV. From electrolyzed solutions at 1500 mV, it is not possible to identify the oxidation product(s). However, a blue film on the electrode surface was observed.

In summary, the results obtained by the GC-MS technique permit us to identify the pyridine derivatives as the final products of the electrolytic oxidation of 4-indolyl-substituted DHPs at an applied potential corresponding to the first oxidation peak. There is no conjugative interaction between the two redox active groups, probably the interaction is of inductive type.

*Reactivity toward alkylperoxyl radicals ABAP-derived.*— According to the above electrochemical results in both media, we can conclude that an intramolecular interaction occurs between the 1,4-DHP and indolyl moieties, thus producing a significant effect on the oxidation of each redox center. In fact, the presence of the indolyl moiety makes the oxidation of the DHP moiety easier and, improves its antioxidant capabilities. Consequently, to check the antioxidant capabilities of the molecules, the reactivity toward alkylperoxyl radicals ABAP-derived was studied.

The reactivity of synthesized 1,4-DHPs with alkylperoxyl radicals ABAP-derived in aqueous medium at pH 7.4 was followed by UV/vis spectroscopy and GC-MS techniques. The original UV/vis absorption bands between $\lambda = 356$ and 360 nm were followed to assess the reactivity. Results of these experiments revealed that these absorption bands decreased with time in the presence of free radicals (Fig. 7A and B).

In the range of 20–120 μM 1,4-DHP concentrations, all the derivatives exhibited a linear concentration dependence. Figure 7C shows typical linear ln[concentration]-time plots for the reactivity of both indolyl-substituted 1,4-DHPs at a initial concentration of 100 μM. The signal that appears at 280 nm corresponds to the oxidized derivative, that is, the pyridine derivative, which agrees with previous observations.[16] To compare the reactivity, kinetic rate constants of tested 1,4-DHP derivatives and of commercial compounds were used (Table II). From Table II, it is apparent that both 4-indolyl-substituted species are five- to six fold more reactive than nisoldipine, a well-known 1,4-dihydropyridine. Consistently, the synthesized compounds that exhibited the less positive oxidation peak potentials are the most reactive derivatives (Table II).

At the end of the experiments, samples were injected into the chromatograph. Results of these analyses confirmed that after the reaction between the alkylperoxyl radicals and the tested 1,4-dihydropyridines, these latter derivatives are oxidized to pyridines. The fragmentation pathways did not differ from those of electrolyses; that is, the fragments contained the pyridine nucleus, being the most abundant fragment ($m/z$ 366).

## Discussion

From the results, it is clear that the inclusion of an indole group in the four position of a dihydropyridine ring significantly facilitates the oxidation of this latter moiety when compared to the corresponding C4 phenyl-substituted derivative. In an aprotic medium, this compound is oxidized at ~88 mV more positive potential (Table I). However, in this same electrolytic medium, the difference between the oxidation peak potentials corresponding to the dihydropyridine ring is even larger when compared to commercial, such as, nimodipine, which is oxidized at 1240 mV (determined in the present article). This means that this derivative is oxidized at 280 mV more anodic potentials. Furthermore, the difference in the oxidation peak potentials corresponding to the dihydropyridine ring between 4-hydroxyphenyl-substituted 1,4-DHPs and 4-indolyl-substituted 1,4-DHPs is about 80–100 mV under the same experimental conditions.[16]

In conclusion, both redox centers (indole and dihydropyridine ring) are mutually interacting in their redox responses. The interaction produces significant effects on the oxidation of each redox center, making oxidation of the DHP ring easier.

Results of Table I reveal that the generated pyridine is an electron-withdrawing group, turning the oxidation of the indole

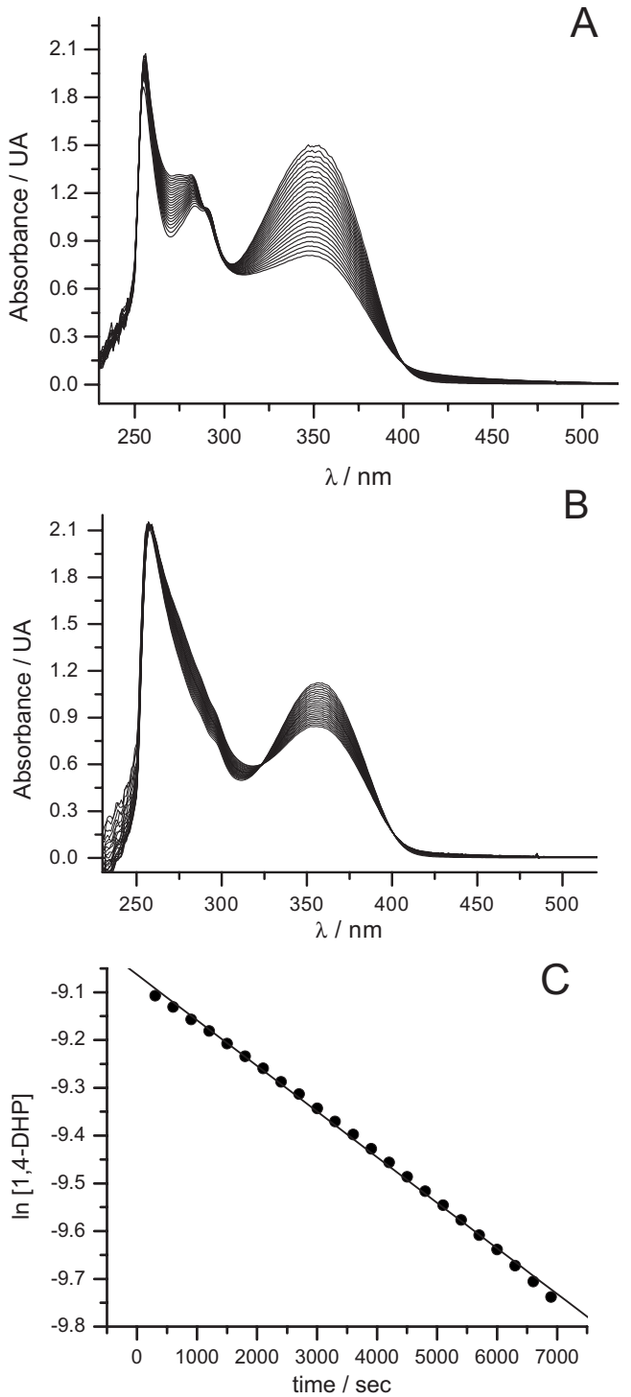


**Figure 7.** (A): UV/Vis spectra for the reaction between 4-(3-indolyl)-DHP and ABAP-derived alkylperoxyl radicals. (B): UV/vis spectra for the reaction between 4-phenyl-DHP and ABAP-derived alkylperoxyl radicals followed during 120 min. Reaction medium: 0.04 M Britton–Robinson buffer/DMF (70/30), pH 7.4 at 37°C. (C) Plot of ln[concentration] vs time for 100 μM 4-(5-indolyl)-DHP in the presence of 100 mM ABAP-derived alkylperoxyl radicals during 120 min at 37°C.

more difficult. It is evident from these results that the inclusion of an indole group as substituent at the C-4 position of the dihydropyridine ring produces a significant increase of reactivity toward alkylperoxyl radicals compared to commercial 1,4-dihydropyridines.

**Table II. Apparent kinetic rate constants for the reaction between C-4-substituted 1,4-DHPs and alkylperoxyl radicals ABAP-derived in Britton–Robinson buffer 0.04 M/DMF; 70/30 at pH 7.4 at 37°C and their relationship.**

| Derivative | $k/10^{-3}/s^{-1}$[a] | $k$/Nisoldipine[b] | Ep[c] |
|---|---|---|---|
| 4-phenyl-DHP and | 0.05 ± 0.005 | 2.5 | 644 |
| 4-(3-indolyl)-DHP | 0.12 ± 0.004 | 6.0 | 596 |
| 4-(5-indolyl)-DHP | 0.11 ± 0.008 | 5.5 | 648 |
| Nisoldipine | 0.02 ± 0.009 | 1.0 | 681 |
| Amlodipine | 0.03 ± 0.007 | 1.5 | 725 |
| Nimodipine | 0.03 ± 0.005 | 2.0 | 736 |

[a] Apparent kinetic rate constant values were calculated from plot ln[DHP] vs time for the reaction toward alkylperoxyl radicals. Values correspond to the average of five independent experiments.
[b] Ratio between apparent kinetic rate constants of the tested 1,4-DHP derivatives/apparent kinetic rate constant of nisoldipine for the reaction with alkylperoxyl radicals in the same experimental conditions.
[c] Oxdation peak potentials obtained by DPV in protic medium (Britton–Robinson buffer/ethanol: 70/30 at pH 7.4.

## Acknowledgments

This work was supported by grants from FONDECYT (grant no. 1050761) and BECA AT (grant no. 23070093).

*Universidad de Chile assisted in meeting the publication costs of this article.*

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