Voltammetric oxidation of Hantzsch 1,4-dihydropyridines in protic and aprotic media: relevance of the substitution on N position

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Abstract

A detailed investigation on the electrochemical oxidation of some Hantzsch 1,4-dihydropyridine derivatives with the aim of study the influence of the hydrogen substituent on the N1 position of the heterocyclic ring have been carried out in protic and aprotic media. For this objective we have synthesized two series of compounds wherein the difference was the substituent (H or ethyl) on the N1-position of the heterocyclic ring. Voltammetry, UV–Vis spectroscopy, Controlled potential electrolysis, EPR, ^{1}H NMR and gas chromatography–mass spectrometry techniques in order to obtain evidences for postulate oxidation mechanisms in both protic and aprotic media have been used. Compounds having the ethyl substituent in the N1 position follow an oxidation mechanism obeying the sequence ECE with the second step as the r.d.e. in both, protic and aprotic media, thus producing the corresponding ethyl substituted pyridinium cation. On the other hand compounds having H in the N1 position follow the same ECE sequence only at acidic media. At basic media, the mechanism consisted of a DISP1 scheme in which rate determining step (r.d.s.) is the uptake of the proton in the N1 position by the OH^- ion of the media. In aprotic media both type of compounds follow the same ECEC mechanism with the second step as the r.d.s. but only the H-substituted compounds generates an anionic species that is more easily oxidized than the parent compounds.

Keywords: 1,4-Dihydropyridines; Oxidation; Voltammetric; *N*-Ethyl-1,4-dihydropyridines

1. Introduction

4-Substituted Hantzsch 1,4-dihydropyridines (1,4-DHP) are analogues of NADH coenzymes [1] and an important class of drugs which are potent blockers of calcium channels with relevant applications in various cardiovascular diseases [2]. 1,4-DHP derivatives undergo rapid and extensive hepatic oxidative metabolization by cytochrome P 450 enzymes [3,4], giving rise to pyridine analogues that are pharmacologically inactive [5,6]. Therefore, the oxidation of 1,4-DHP has recently attracted more attention from chemists [7].

From the structure–activity relationship studies has been possible a best understood about the interaction between calcium channels and the varied chemical structures behaving as calcium blockers. Particularly, for optimal activity of 1,4-DHP as antagonists, the following essential moieties have been detected: (a) the presence of the intact 1,4-DHP ring; (b) the secondary nitrogen in the heterocyclic; (c) a space-filling substituent in *para*-position of the 1,4-DHP ring; (d) the presence of ester groups in 3- and 5-position and the methyl groups in 2- and 6-position on the 1,4-DHP ring also result relevant to conserve the antagonist effects of the derivatives [8].

The oxidation of 1,4-DHP is one of the ubiquitous problems in organic chemistry, and several researchers have reported oxidation methods including chemical oxidation with ferric or cupric nitrates on a solid support [9], oxidation with ceric ammonium nitrate [10], ultrasound-promoted oxidation by clay-supported cupric nitrate [11], oxidation with pyridinium chlorochromate [12] and oxidation with nitric acid [13]. More recently the oxidation of 1,4-DHP derivatives has

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attracted more attention from chemists because of its oxidative reactivity with endobiotics such as nitric oxide (NO) or its donor *N*-methyl-*N*-nitrosotoluene-*p*-sulfonamide (MNTS) and superoxide ($O_2^{\bullet -}$) [14–17].

From the electrochemical point of view the oxidation of some 1,4-DHP have previously been performed in both non-aqueous and aqueous media using mainly rotating ring disk electrode, linear and cyclic voltammetry (CV), and ESR spectroscopy. The more detailed mechanistic information has been obtained in non-aqueous solvents [18–22]. The first stage of electrooxidation of 1,4-DHP consists of removal of one electron from the starting 1,4-DHP molecule with formation of a cation radical, 1,4-DHP$^{\bullet +}$. This cation radical becomes deprotonated at a high rate forming a neutral radical, Py$^{\bullet}$, which oxidizes further (at the same or minor potential of the first stage), to produce pyridinium cations, Py^{+}, and then deprotonated to form the pyridine derivative. The decay of the primary product, cation radical, 1,4-DHP$^{\bullet +}$, seems to be the key stage of the electrooxidation process and two ways of the decay of these cation radicals can occur, i.e. via loss of H^+ or via loss of $H^{\bullet}$ have been proved to be possible. Competition between these two ways depends on the influence of temperature and on electronic effects of the substituents attached to the 1,4-DHP ring. In aqueous media [23–26] 1,4-DHP are reported to undergo a simple two-electron anodic process producing the corresponding pyridine derivative with the concomitant release of protons. However a more deep insight on the mechanism involved in aqueous media was recently carried out by Ruiz Montoya et al., [27,28]. These authors studied the oxidations of diethyl 1,4-dihydro-2,4,6-trimethyl-3,5-piridinedicarboxylate and 1,4-dihydro-2,6-dimethyl-3,5-piridinedicarboxylate finding that in acidic media, the rate determining step (r.d.s.), was the deprotonation of the radical cation formed after the first one-electron transfer. In basic media, the mechanism consisted of a DISP1 scheme in which r.d.s. is the uptake of an H^+ ion by both the OH^- ion and the basic components of the buffer, for the first compound and only by the OH^- ion, for the second compound.

The state of the art concerning the electrochemical oxidation of substituted 1,4-DHP revealed the importance of some substituents, such as in the N1 and C4 position of the 1,4-DHP ring. In fact, the stability of the cation radical appear to be strongly affected by these substituents. In a previous work [25] the influence of different substituents at the C4-position but maintaining H at the N1-position was studied. In that work a direct correlation between the easy of oxidation with the electron donating character of the substituent was obtained. In the present paper we have synthesized three 1,4-DHP derivatives wherein the H at the N1-position was replaced by an ethyl group with the objective to study the influence of the H at the N1-position in the electrochemical oxidation mechanism. The evidence obtained in recent previous works wherein the H at the N1-position results to be crucial in the oxidative reactions of 1,4-DHP with ABAP-derived alkyl radicals [25] and superoxide anion [17] have increased the interest in studying the role of the H–N1 in the electrochemical oxidation of 1,4-DHP.

2. Experimental

2.1. Compounds

All compounds (Fig. 1) were synthesized in our laboratory according to previous work [29].

2.1.1. Series a (compounds *I–III*)

General procedure: A mixture of 0.079 mol of methyl acetoacetate and 10 ml of concentrated ammonium hydroxide in 20 ml of ethyl alcohol was heated under reflux for 2.5 h. The resulting clear solution was added to a mixture of 0.079 mol of methyl acetoacetate, 0.165 mol of the corresponding aldehyde (Compound **I** = acetaldehyde; Compound **II** = 4-methoxybenzaldehyde; Compound **III** = 4-nitrobenzaldehyde), 25 ml of concentrated ammonium hydroxide and 20 ml of ethyl alcohol and maintained under reflux for 10 h. The crude solid product was filtered and recrystallized in ethyl alcohol. The yields were in the 80–90% range depending upon the derivative. The following 1,4-DHP derivatives were synthesized following the just described general procedure.

*2.1.1.1. 4-Methyl-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine (**I**).* IR (KBr): ν_{max} 3342, 2950, 1680, 1650, 1435, 1351, 1226, 1056 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, 3H, $J = 6.5$ Hz, $>CH-CH_3$), 2.29 (s, 6H, $-CH_3$), 3.73 (s, 6H, $-O-CH_3$), 3.83 (q, 1H, $J = 6.5$ Hz, $>CH-CH_3$), 5.73 (s, 1H, $-NH-$) ^{13}C NMR (75 MHz, $CDCl_3$): (20.35 × 2), 23.20, 29.30,

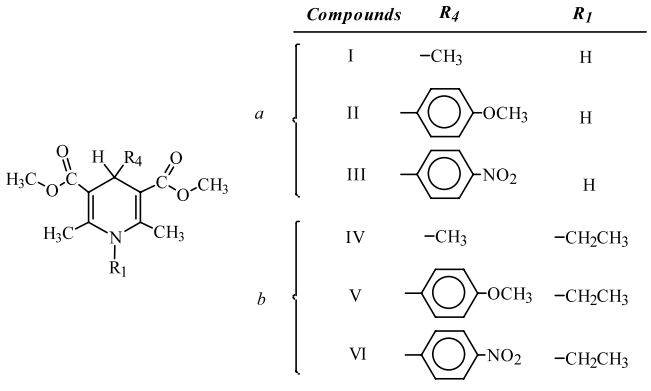


Fig. 1. Chemical structures of 1,4-DHP belonging to serie: (a) with and (b) without an H substituent on the N1.

(51.92 × 2), (105.27 × 2), (145.64 × 2), (169.19 × 2). *Anal.* Calc. for $C_{12}H_{17}O_4N$: C, 60.25; H, 7.13; N, 5.86. Found: C, 60.27; H, 7.23; N, 5.87%. M.p.: 147–149 °C.

*2.1.1.2. 4-(4-Methoxyphenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine (**II**).* IR (KBr): ν_{max} 3349, 2949, 1697, 1650, 1431, 1383, 1251, 1213, 1027 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.33 (s, 6H, $-CH_3$), 3.65 (s, 6H, $-O-CH_3$), 3.75 (s, 3H, Ar–O–CH_3), 4.94 (s, 1H, Ar–CH<), 5.76 (s, 1H, –NH–), 6.75 (d, 2H, $J = 8.6$ Hz, Ar–H), 7.2 (d, 2H, $J = 8.6$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$): (19.51 × 2), 38.31, (50.94 × 2), 55.05, (104.00 × 2), (113.30 × 2), (128.53 × 2), 139.86, (143.93 × 2), 157.85, (168.06 × 2). *Anal.* Calc. for $C_{18}H_{21}O_5N$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.00; H, 6.47; N, 4.36%. M.p.: 181–183 °C.

*2.1.1.3. 4-(4-Nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine (**III**).* IR (KBr): ν_{max} 3343, 2948, 1703, 1655, 1518, 1434, 1384, 1347, 1218, 1020 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 6H, $-CH_3$), 3.66 (s, 6H, $-O-CH_3$), 5.12 (s, 1H, Ar–CH<), 5.85 (s, 1H, –NH–), 7.46 (d, 2H, $J = 8.8$ Hz, Ar–H), 8.12 (d, 2H, $J = 8.8$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$): (20.63 × 2), 40.73, (52.11 × 2), (103.92 × 2), (124.39 × 2), (129.53 × 2), (145.81 × 2), 147.29, 155.64, (168.38 × 2). *Anal.* Calc. for $C_{17}H_{18}O_6N_2$: C, 58.96; H, 5.24; N. 8.09. Found: C, 58.76; H, 5.03; N, 8.25%. M.p.: 165–168 °C.

*2.1.2. Series b (compounds **IV**–**VI**)*

The synthesis was carried out by *N*-alkylation with iodide halogenure of each 4-substituted 1,4-DHP.

General procedure: 0.004, 0.003 and 0.0029 mol of compound **I**, **II** and **III**, respectively, mixed with 0.0125 mol of sodium hydrure and 0.0124 mol of ethyl iodide in 20 ml tetrahydrofurane. Solutions were bubbled with Argon for 15 min and then incubated at room temperature keeping constant agitation by 10 h. The obtained solutions were filtered in vacuum and the liquid was extracted with chloroform three or four times. Chloroform extracts were evaporated and the solid products were recrystallized in ethyl alcohol. The yields were in the 30–40% range depending upon the derivative. The following 1,4-DHP derivatives were synthesized following the just described general procedure:

*2.1.2.1. 4-Methyl-2,6-dimethyl-3,5-dimethoxycarbonyl-N-ethyl-1,4-dihydropyridine (**IV**).* IR (KBr): ν_{max} 2954, 1696, 1631, 1434, 1389, 1212, 1163, 1056 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, 3H, $J = 6.6$ Hz, >$CHCH_3$), 1.16 (t, 3H, $J = 7.1$ Hz N–CH_2CH_3), 2.4 (s, 6H, $-CH_3$), 3.7 (q, 2H, $J = 7.1$ Hz N–CH_2CH_3), 3.72 (s, 6H, $-OCH_3$), 3.77 (q, 1H, $J = 6.6$ Hz, >$CHCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): 15.56, (16.15 × 2), 21.73, 28.12, 39.51, (51.12 × 2), (108.75 × 2), (148.01 × 2), (168.50 × 2). *Anal.* Calc. for $C_{14}H_{21}NO_4$: C, 62.84; H, 7.85; N, 5.23. Found: C, 63.05; H, 7.62; N, 5.13%. M.p.: 78–80 °C.

*2.1.2.2. 4-(4-Methoxyphenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-N-ethyl-1,4-dihydropyridine (**V**).* IR (KBr): ν_{max} 2948, 1690, 1629, 1433, 1390, 1256, 1213, 1152, 1035 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.06 (t, 3H, $J = 5.9$ Hz, NCH_2-CH_3), 2.47 (s, 6H, $-CH_3$), 3.67 (q, 2H, $J = 5.9$ Hz, $-N-CH_2CH_3$), 3.7 (s, 6H, $-OCH_3$), 3.75 (s, 3H, Ar–OCH_3), 5.04 (s, 1H, Ar–CH<), 6.75 (d, 2H, $J = 8.7$ Hz, Ar–H), 7.09 (d, 2H, $J = 8.7$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.06, (16.42 × 2), 37.19, 40.42, (51.26 × 2), 55.17, (107.16 × 2), (113.32 × 2), (127.91 × 2), 138.53, (148.53 × 2), 157.90, (168.61 × 2). *Anal.* Calc. for $C_{20}H_{25}NO_5$: C, 66.77; H, 6.96; N, 3.9. Found: C, 66.85; H, 7.08; N, 3.66%. M.p.: 106–108 °C.

*2.1.2.3. 4-(4-Nitrophenyl)-2,6-dimethyl-3,5-dimethoxycarbonyl-N-ethyl-1,4-dihydropyridine (**VI**).* IR (KBr): ν_{max} 2945, 1690, 1624, 1511, 1382, 1345, 1252, 1154, 1025 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.04 (t, 3H, $J = 7.1$ Hz, $N-CH_2CH_3$), 2.5 (s, 6H, $-CH_3$), 3.72 (s, 6H, $-OCH_3$), 3.72 (q, 2H, $J = 7.1$ Hz, $N-CH_2-CH_3$), 5.19 (s, 1H, Ar–CH<), 7.34 (d, 2H, $J = 8.5$ Hz, Ar–H) 8.07 (d, 2H, $J = 8.5$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.06, (16.4 × 2), 38.25, 40.0, (51.44 × 2), (105.70 × 2), (123.29 × 2), (127.74 × 2), (146.42 × 2), 149.32, 153.61, (167.94 × 2). *Anal.* Calc. for $C_{19}H_{22}N_2O_6$: C, 60.9; H, 5.88; N, 7.48. Found: C, 61.10; H, 5.99; N, 7.34%. M.p.: 156–157 °C.

NMR spectroscopy: the NMR spectra were recorded on a Bruker spectrometer advance DRX 300. FT-IR: The IR spectra were recorded on a Bruker spectrometer IFS 55 Equinox. Elemental analyses were performed in a Fisons Instrument equipment.

2.2. Electrolytic media

The protic media consisted in a Britton–Robinson buffer 0.04 M with 30% ethanol. For aprotic media acetonitrile (AN) (Merck) was used.

For all compounds, concentrations between 0.05 and 1 mM in 0.1 M KCl (Merck) or 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP) (Sigma) for protic or aprotic media were used, respectively. The experiments were carried out under an atmosphere of pure and dry nitrogen.

2.3. Voltammetry

Differential Pulse (DPV), CV and linear sweep voltammetry were performed with a BAS CV50 assembly. A glassy carbon stationary electrode as working electrode for DPV and CV experiments was used. For dynamic experiments a glassy carbon rotating disk

electrode was also employed. A platinum wire was used as counter electrode and all potentials were measured against an Ag/AgCl electrode.

2.4. Controlled potential electrolysis

CPE were carried out on a platinum mesh electrode in anhydrous acetonitrile 0.1 M TBAHFF. For protic media CPE were carried on a glassy carbon mesh electrode. 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. The net charge was calculated by correction for the estimated background current.

2.5. Electrolysis—EPR

The EPR 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. The electrolysis was performed in same conditions as described above. The concentration of the compounds was 1 mM. The concentration of the spin trap, *N*-tert-butylamine-α-phenylnitrone (PBN) was at least 100 times higher.

2.6. UV–Vis spectrophotometry

Spectra were registered using an UNICAM UV-3 spectrophotometer. UV–Vis spectra were recorded in the 200–400 nm range at different intervals. Acquisition and data treatment were carried out with vision 2.11 software.

2.7. Gas chromatography–mass spectrometry

2.7.1. Equipment

A Gas Chromatograph/Mass Selective Detector 5890/5972 Detector (Hewlett-Packard, Palo Alto, CA) and Hewlett-Packard 7673 Autosampler were used for the analyses. A Hewlett-Packard Data System based on a Pentium II processor printer was used to control instrumentation and for data processing. The m/z range monitored was 45–550 with a scan rate of 1 scan s^{-1}; the normal energy electron was set at 70 eV.

2.7.2. Chromatography column

A Hewlett-Packard Ultra-1 column, 25 m × 0.2 mm i.d. × 0.11 μm film thickness (Little Falls, Wilmington, DE), was used.

2.7.3. 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}.

2.7.4. 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). After controlled potential electrolysis, the final solutions were diluted and injected without any class of previous treatment. Consequently no extraction or filtration procedures were necessary.

2.8. Reactivity with potassium superoxide

A ^{1}H NMR spectra of 40 mM of compound **II** in DMSO-d_6 (solution A) was registered. Then the spectra of solution A plus 60 mM of KO_2 in 0.1 M Crown ether/18-crown 6 (Merck) (solution B) was registered.

3. Results and discussion

All the synthesized compounds **I**–**VI** were electrochemically oxidized on glassy carbon electrode in both protic (aqueous–alcoholic) and aprotic (non-aqueous) media.

3.1. Protic media

All the compounds displayed a very well defined oxidation peak when submitted to a DPV experiment on a glassy carbon electrode. The electrooxidation process of **I**–**III** derivatives was pH-dependent at pH >4 but at acidic pHs between 1 and 4 was pH-independent, as was showed in a previous paper [25]. Furthermore those experiments showed that 4-substitution strongly affects the oxidation of the 1,4-DHP. In fact we concluded that in the case of compounds **I**–**III**, meanwhile more electron donating character of the 4-substituent more easy was the oxidation of the 1,4-DHP. On the other hand, when compounds **IV**–**VI** were submitted to DPV experiments, surprisingly a totally different behavior obtaining pH-independent peaks in all the pH range was observed. The comparative pH-behavior between compounds **I**–**III** (with a secondary amine in the 1,4-DHP) and compounds **IV**–**VI** (with a tertiary amine in the 1,4-DHP) is shown in Fig. 2. As can be ascribed from these results, at acidic pHs <4, all compounds are oxidized independently of pH meaning that protons are not involved in the process before the r.d.s. In the case of compounds **IV**–**VI**, that lacking a H substituent in position N1, the pH-independence was maintained at all

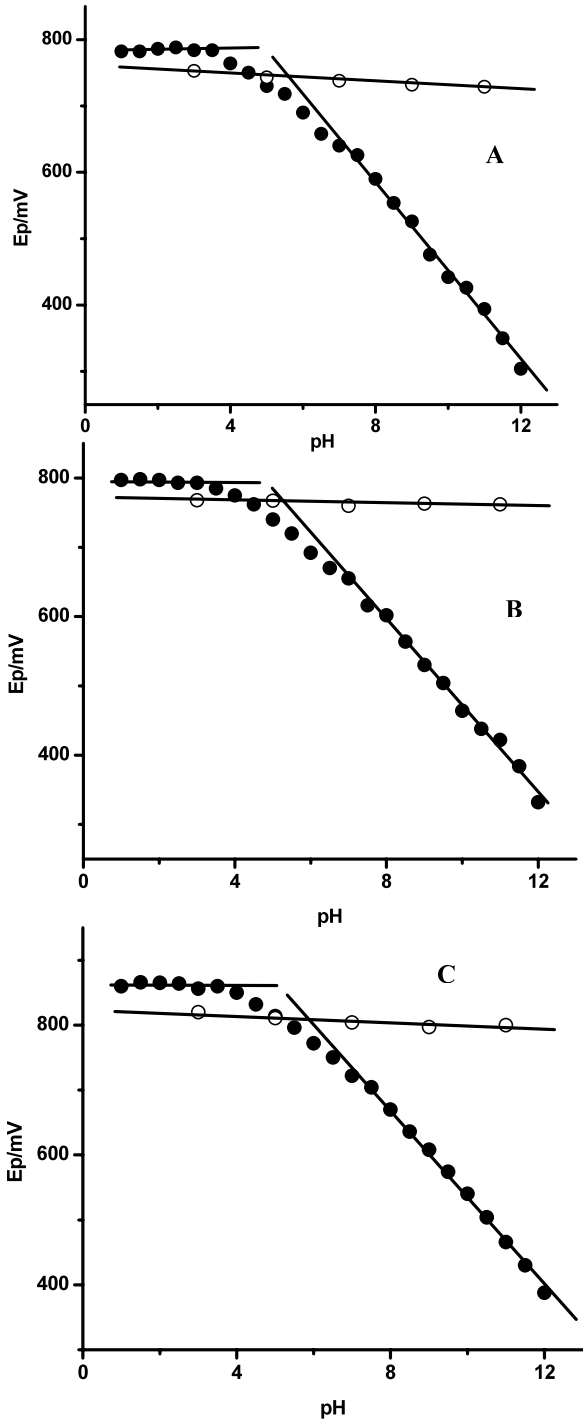


Fig. 2. Comparative dependence of peak potentials with pH for different 1,4-DHP with and without an H atom on the N1 position, in protic media. (A) Compounds **I**, **IV**, (B) **II**, **V** and (C) **III**, **VI**.

pH conditions showing a markedly difference with compounds **I**–**III**. In fact, according to this behavior, compounds **IV**–**VI** are oxidized according to the same mechanism in all the pH range and the mechanism do not involve proton transfer before the r.d.s. Consequently we can assume that all the studied compounds **I**–**VI** follow a same pH-independent mechanism only at pH <4. On the other hand when we compare the oxidation potentials belonging to both series of compounds, in the pH <4 zone, we found that compounds having H as substituent in N1-position are more difficult to oxidize when compared with the corresponding analog compounds but having an ethyl substituent in N1. These results are in accord with the fact that the ethyl substituents in N1-position have a greater electron donating effect than the H substituent thus producing an enhanced electron density on the dihydropyridine ring facilitating the oxidation process. At pH >4 both types of compounds follow different mechanisms and securely the difference is due to the presence of the H substituent in N1. In Table 1 we have summarized the behavior of the peak potential at three different pHs. If we compare the results at pH 2, wherein a pH-independent mechanism for all compounds is followed, we can observe two main aspects: (a) comparison between compounds belonging to the same series (i.e. **I**–**III** or **IV**–**VI**) shows peak potential differences between them that can be explained with the different substitution in C4; and (b) comparison between compounds belonging to different series (i.e., **I** with **IV**, **II** with **V** and **III** with **VI**) shows peak potential differences between them that can be explained according to the substitution on N1. At pH >4 we can observe that both series of compounds follow different mechanisms but the presence of H substituent in N1 produces more oxidizables compounds portending that the proton on the amine secondary group of the 1,4-DHP would play an important role in the facilitation of the oxidation process. At pH >8 the slopes of the E_p vs pH behavior were 68.9, 62.5 and 71.1 mV per pH unit for compounds **I**, **II** and **III**, respectively.

In order to study the dependence of the limiting current with pH we prefer a dynamic voltammetry wherein a steady state is attained rather quickly and, measurements can be made with high precision. Moreover, at steady state, double-layer charging does not enter the measurement [30]. In fact, we have carried out measurements using a glassy carbon rotating disk

Table 1
Peak potential values obtained for 1,4-DHP derivatives at three different pHs in protic and aprotic media

Compound	E_p/mV vs Ag/AgCl			
	pH 2	pH 7.4	pH 11	Aprotic medium
I	786	640	394	948
II	805	655	422	994
III	870	722	466	1093
IV	743	738	729	915
V	768	760	762	933
VI	820	824	–	1036

Values were obtained from differential pulse voltammetry experiments on stationary glassy carbon electrode.

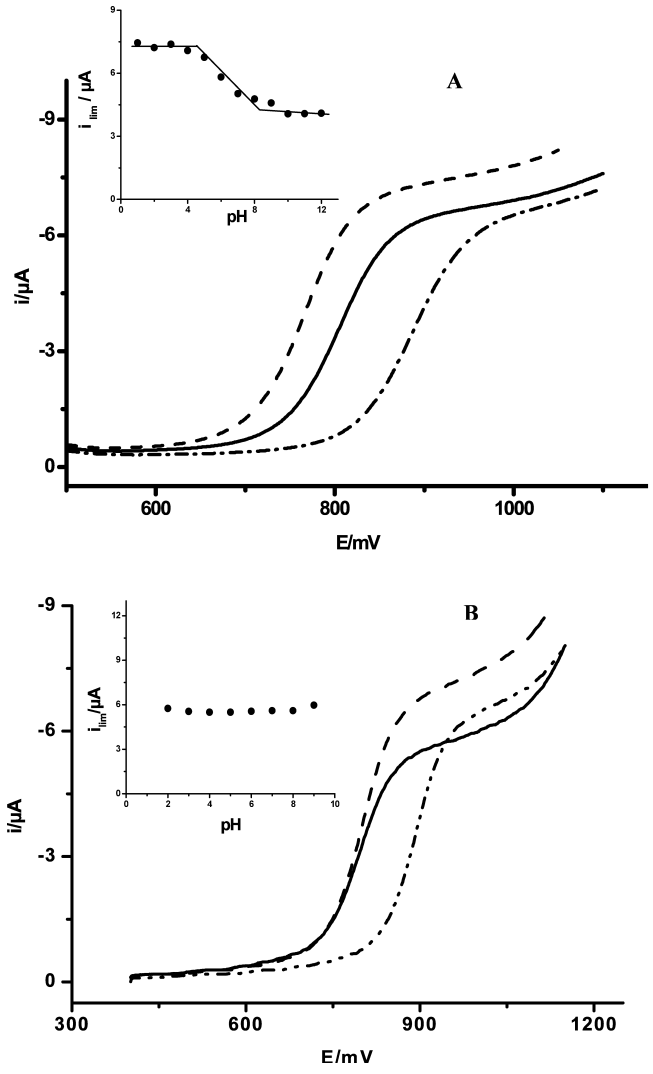


Fig. 3. Dynamic voltammograms on glassy carbon electrode in protic media at pH 5. (A) Compounds (---) **I**, (—) **II**, and (-·-) **III**. *Insert:* Dependence of limiting current with pH for a 100 µM concentration of compound **I** in protic media. (B) Compounds (---) **IV**, (—) **V**, and (-·-) **VI**. *Insert:* Dependence of limiting current with pH for a 100 µM concentration of compound **IV** in protic media. Protic media: 0.04 M Britton Robinson buffer+ethanol: 70/30. Rotating rate 5.200 rpm.

electrode to obtain reproducible voltammograms at different pHs. In Fig. 3A and B we can observe the dynamic voltammograms obtained at pH 5 for compounds **I**–**III** and **IV**–**VI**, respectively. Moreover in the inserts of Fig. 3A and B we can observe the evolution of the limiting current with pH for both serie of compounds. The results are totally consistent with the above obtained from the peak potential pH-dependence. In fact, the limiting current for compounds **IV**–**VI** are pH-independent claiming a diffusion controlled process for this type of compounds. In the case of the **I**–**III** compounds, a lowering to a half of the limiting current on passing from pH <4 to pH >8 was observed. This lowering is suggestive of the subtraction of half electroactive species or its conversion by electrodimerization or a disproportionation.

The obtained behavior of both the limiting current and peak potential with pH was exactly the same that has been reported previously for two related 1,4-DHP derivatives [27,28]. In those articles was concluded that in acidic media the r.d.s., was the deprotonation of the radical cation formed after the first one-electron transfer, but in basic media the mechanism consisted of a DISP1 scheme in which r.d.s. is the uptake of an H^+ ion by both the OH^- ion or the basic components of the buffer. A similar DISP1 mechanism has also been found for the reaction of NADH analogues with bases [31–33]. Consequently a DISP1 mechanism as in such previous articles will be assumed for compounds **I**–**III** in protic media at basic pHs.

Cyclic voltammograms carried out with all the studied compounds show only one irreversible anodic wave at different sweep rates (0.1–10 V s^{-1}). In all the cases, $\log I_p$ vs $\log v$ plots exhibited slopes close to 0.5, indicating that no adsorption processes are involved. Furthermore, the peak potential values (E_p) were dependent on the sweep rates with $\delta E_p/\delta \log v$ values of 39.3, 28.1 and 31.5 for compounds **I**–**III**, respectively.

UV–Vis spectra in the region 200–400 nm at different pH values were recorded for all the compounds, exhibiting similar maxima about 240 and 350 nm. However these maxima were not altered with pH changes suggesting that there were no reasons to think that the electroactive species could change in these media.

In order to determine the number of electrons involved in the oxidation process, coulommetric analysis were carried out in aqueous media at pHs 2 and 11. Solutions containing accurately weighed amounts of all the 1,4-DHP derivatives in aqueous media were subjected to electrolysis at constant applied potential and the charge passed was registered. The applied potentials for compounds **I**–**III** were between 850 and 950 mV at pH 2 and 400–480 mV at pH 12. For compounds **IV**–**VI**, the applied potential was between 800 and 900 mV at pH 2 and 12. A comparative coulommetric analysis for compounds with and without H at N1 position is shown in Fig. 4. From these curves the number of electrons transferred in the oxidation process was calculated. The results in Table 2 reveal that compounds **IV**–**VI** are oxidized according to a two-electron oxidation process in all the pH range. However compounds **I**–**III** consume two electrons at acidic pH (pH 2) and only one electron at basic pH (pH 11). These results are consistent with the lowering to a half of the limiting current on passing from pH <4 to pH >8 observed in the voltammetric experiments for compounds **I**–**III**. This finding also supports the assumed DISP1 mechanism for this type of compounds in basic media.

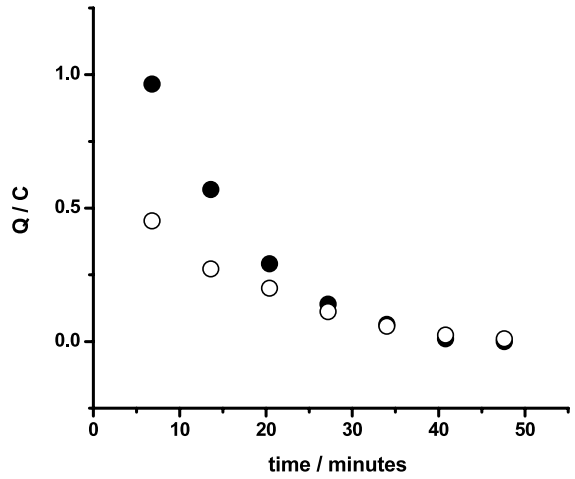


Fig. 4. Charge consumed during the controlled potential electrolysis of 1 mM of compound **II** at pH 2 (○) and pH 11(●).

Furthermore, as was proved in a previous paper [34], the electrochemical oxidation of the H–N1 substituted 1,4-DHP monitored by gas chromatography–mass spectrometry (GC–MS) revealed the formation of the corresponding pyridine derivatives. However in the case of the *N*-ethyl substituted compounds the formation of pyridinium salts instead of the pyridine derivative was attained. In order to obtain further evidences supporting a DISP1 mechanism in protic media for compounds **I**–**III** we have integrated CPE generation of products with GC–MS identification of them. When compounds **I**–**III** were submitted to exhaustive electrolysis at pH 2, a 100% of pyridine derivative was attained as the only product. On the other hand, when exhaustive CPE was carried out at pH 11 we have obtained only 86% conversion to the pyridine derivative with a 14% of the parent 1,4-DHP as other product of the electrolysis. This fact was indicative that in basic media the 1,4-DHP derivative is also generated in the electrolysis supporting a DISP1 mechanism.

3.2. Aprotic media

DPV was also carried out in aprotic media (AN with 0.1 N TBAHFP); results of these experiments did not differ from those in protic media, i.e. only a single peak was observed. In Table 1 the peak potential values both in protic and aprotic media are shown. As can be seen the ease of oxidation of all compounds is favored in protic media. From these results in aprotic media we can generalize that *N*-ethyl substituted compounds are more easily oxidized than compounds lacking the H substituent in position N1. On the other hand compounds belonging to the same series (i.e. **I**–**III** or **IV**–**VI**) show peak potential differences between them that can be explained with the different substitution in C4 in a similar way than protic media.

All the compounds produced very well resolved voltammetric curves when submitted to dynamic voltammetry on a glassy carbon rotating disk electrode. Furthermore, all the compounds were submitted to CV wherein only one irreversible oxidation peak was evident. With the objective to achieve higher sweep rates a glassy carbon microelectrode was used. Surprisingly when sufficiently high sweep rates (200 V s^{-1}) were attained, a cathodic peak was insinuated in the reverse sweep but quantitative measurements with the couple was impossible (Fig. 5). Probably this cathodic peak reveals the reduction of a relatively unstable specie (cation radical or pyridinium radical) formed in the forward sweep. This is the first voltammetric view wherein the appearance of a radical specie was evidenced by CV. Obviously this view is only obtained in aprotic media. Furthermore this evidence of a radical specie was attained from both type of compounds i.e. with and without H substituent in N1, however appar-

Table 3
Splitting constants of the resulting spin adducts of 1,4-DHP compounds

Compound	a_N/G	a_H/G
I	14.0	3.4
II	13.9	2.8
III	13.8	2.5
IV	14.6	3.5
V	14.1	3.2
VI	13.9	2.9

Table 2
Apparent number of electrons transferred obtained by CPE for the oxidation of 1,4-DHP in both protic (acid and basic) and aprotic media

Compound	Number of electrons		
	Aqueous medium (pH 2)	Aqueous medium (pH 11)	Aprotic medium
I	2.0±0.1	1.2±0.1	1.8±0.1
II	2.1±0.2	1.1±0.2	2.1±0.2
III	1.8±0.1	0.9±0.1	2.2±0.2
IV	2.0±0.2	1.9±0.1	2.0±0.1
V	1.9±0.1	2.1±0.2	2.2±0.3
VI	2.0±0.1	–	2.0±0.1

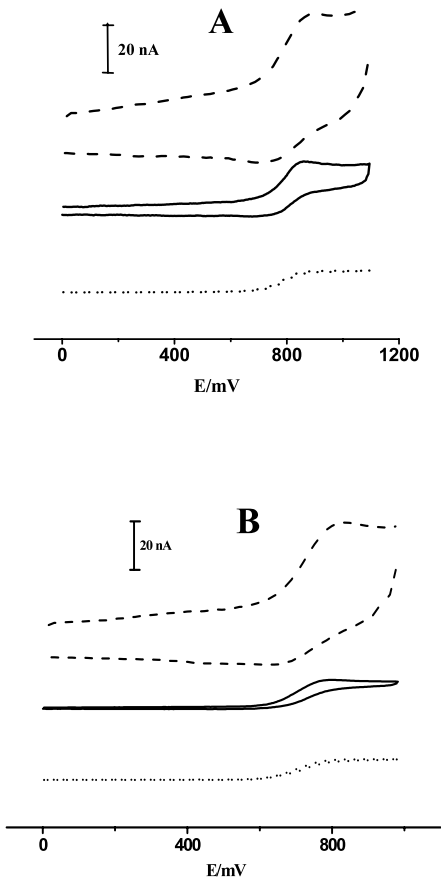


Fig. 5. Cyclic voltammograms on glassy carbon microelectrode at different sweep rates. (A) Compound **I**; (B) Compound **IV**, 2 mM in acetonitrile. (·····) 1 V s^{-1}, (—) 50 V s^{-1}, (---) 250 V s^{-1}.

ently the radical appear to be more stabilized in compounds having the H–N1 moiety. The voltammetric evidence of the presence of radical species was confirmed by spin trapping studies with EPR.

In fact according to previous EPR spin trapping studies [25] we have proved that compounds belonging to the series **I**–**III** produce trapped species corresponding to the pyridinium radical. Using the same methodology previously described we have carried out EPR experiments using the spin trap PBN with the objective to trap radical species from the compounds belonging to the series with ethyl substituent on the N1 position. Electrolysis of compounds **IV**–**VI** at 1 mM concentration, in the presence of PBN, gave spectra characteristics of a nitroxide spin adduct (Fig. 6). a_N values around 14 G and a_H splitting values of 2–3 G were obtained for these spin adducts (Table 3). As can be seen the obtained values for compounds **IV**–**VI** did not differ from the values for compounds **I**–**III** proving that the same radical specie was trapped for both type of compounds. Consequently, based on this comparison and the previously informed [25] for compounds **I**–**III**, we can conclude that the radicals generated for all compounds

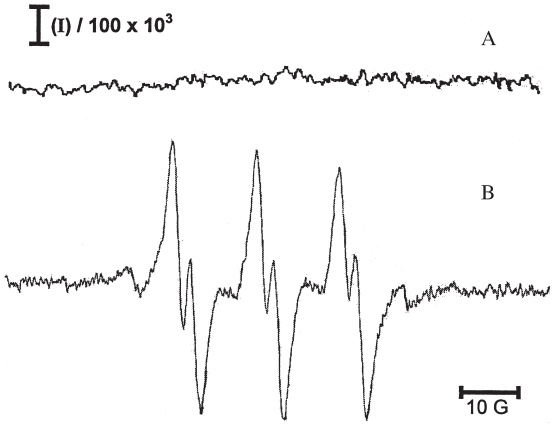


Fig. 6. Experimental EPR spectra of adduct PBN–pyridinium radical. Radical generated electrochemically from 1,4-DHP solution in acetonitrile + 0.1 M TBAHFFP of: (A) base line, and (B) 1 mM compound **IV** + 100 mM PBN (70 scans).

are preferentially added to the spin trap via the reactive C4-position and correspond to the pyridinium radical.

CPE experiments were carried out at controlled potentials between 990 and 1200 mV for compounds **I**–**III** and 900 and 1050 mV for compounds **IV**–**VI**, respectively. The results revealing that two electron are transferred in the overall reaction are showed in Table 2.

One of the most markedly difference between compounds belonging to the **I**–**III** series with those belonging to the **IV**–**VI** series is the feasibility to produce an anionic specie with lost of the proton on the N1. In fact compounds of the **I**–**III** series generated the anionic specie by adding a base (alcoholic NaOH solution) to the aprotic solution containing the 1,4-DHP derivative, as was previously proved using voltammetric and UV–Vis spectrophotometric evidences [25]. In the present study we have reaffirmed the existence of the formation of the anionic specie in the **I-III** series but using a different base as potassium superoxide (KO_2) which permits additional NMR spectroscopic evidences proving the anion formation. From the voltammetric point of view the effect of adding a base as KO_2 resulted in the disappearance of the main signal at approximately 1000 mV in parallel with the appearance of a new signal at approximately 0 mV. This phenomena was reverted with the addition of acid (Fig. 7). The same change can be tracked with UV–Vis spectrophotometry wherein the added base produce the disappearance of the absorption band at approximately 350 nm with the appearance of a new band at approximately 430 nm. Furthermore we have used ^1H NMR in order to confirm the existence of the dihydropyridine anion. In Fig. 8 we have shown a restricted NMR spectra, showing only the most relevant signals, to prove that the H linked to the N1 was lost after adding a base as KO_2 to produce the anionic form of the dihydropyridine derivative. As can be seen from the spectra the adding of KO_2 produce the elimination

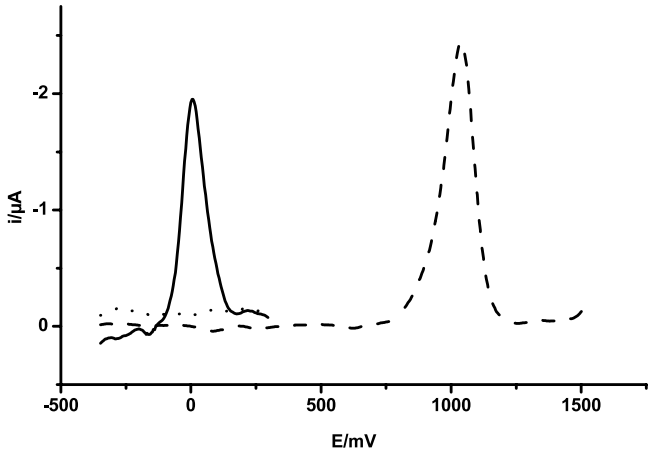


Fig. 7. Differential pulse voltammograms of 100 μM concentration of compound **II** in acetonitrile: (---) original voltammogram, (—) +2 mM KO_2, (...) +1 mM $HClO_4$.

of the signal at 8.8 ppm confirming the lost of the H in the N1 position. On the other hand addition of a base to an aprotic solution containing compounds of the series **IV**–**VI** did not produce any voltammetric, spectrophotometric nor spectroscopy effect confirming that the anion formation is due to the presence of the H on the N1.

Summarizing, 1,4-DHP derivatives having a H substituent on the N1 position are capable to form the corresponding anionic form by reaction with a base. Moreover, this anionic form results to be more easier to oxidize than the parent derivative. Consequently both aspects could be a support explaining the observed reactivity between 1,4-DHP and compounds as superoxide or nitric oxide recently informed [15,17].

3.3. Mechanisms

According to the above results and other published results from related compounds [27,28] some mechanisms concerning the oxidation of the studied 1,4-DHP can be postulated. Our results show that both type of compounds follow different mechanisms revealing the relevance of the substitution on the N atom of the heterocyclic. In the case of compounds belonging to the series **IV**–**VI** which lacking an H substituent on the N1 atom follow a similar mechanism in both protic and aprotic media. The mechanism can be summarized according to the exposed in Scheme 1.

On the other hand, compounds belonging to the series **I**–**III** having an H substituent on the N1 position in protic media, follow different mechanisms if acid or basic media are involved. Our experimental evidences for these compounds in protic media adjust very well with the previously informed for the electrochemical oxidation of other related H–N1 substituted 1,4-DHP [27,28]. Consequently we assumed the same mechanisms which can be summarized in Schemes 2 and 3 depending if acid or basic media are involved, respectively. In acidic media the r.d.s., was the deprotonation of the radical cation formed after the first one-electron transfer. In basic media, the mechanism consisted of a DISP1 scheme in which r.d.s. is the uptake of an H^+ ion mainly by the OH^- ion of the aqueous–alcoholic medium. Compounds belonging to the series **I**–**III** are oxidized, in aprotic medium, following the general mechanism shown in Scheme 4.

4. Conclusions

The H substituent in the N1 position of the 1,4-DHP derivatives affects considerably the electrochemical behavior. In fact compounds having an ethyl group instead the H substituent follow the same mechanism in protic (pH independent mechanism) or aprotic media. However compounds bearing the H substituent follow a

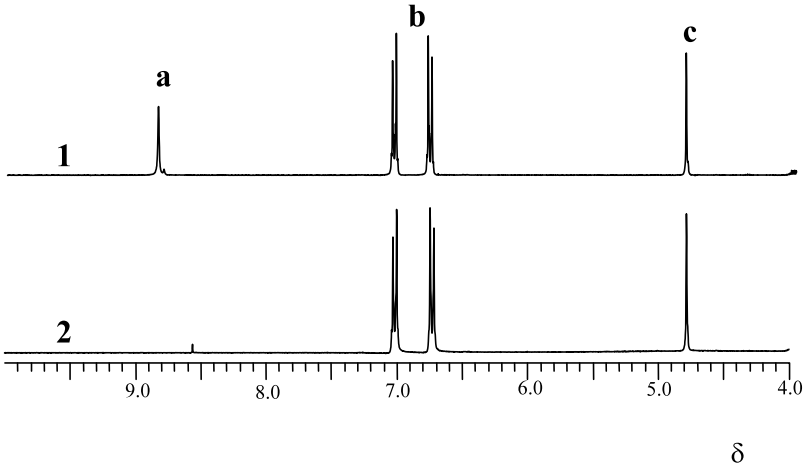
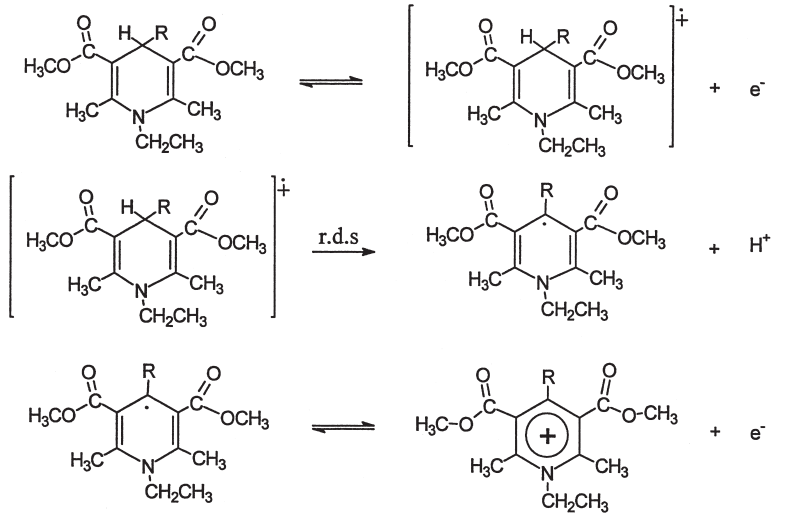


Fig. 8. ^1H NMR spectra of compound **II** en DMSO-d_6. Spectrum 1: without KO_2. Spectrum 2: with KO_2 added. Signal a: hydrogen on N1. Signal b: aromatic hydrogen. Signal c: hydrogen on C4.



Scheme 1. Oxidation mechanism of 1,4-DHP derivatives (**IV**–**VI**) in protic and aprotic media.

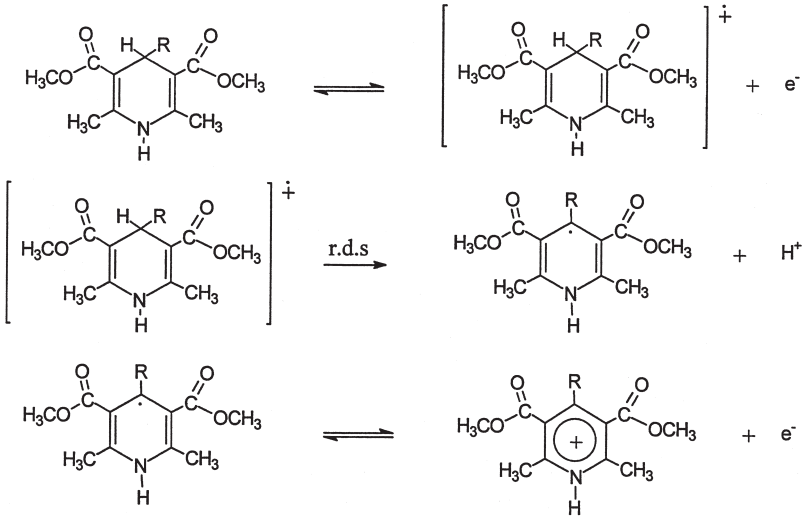
different mechanism depending if acid, basic or aprotic media are involved.

Compounds having the ethyl substituent in the N1 position follow an oxidation mechanism obeying the sequence ECE with the second step as the r.d.e. in both, protic and aprotic media producing the corresponding ethyl substituted pyridinium cation. On the other hand compounds having H in the N1 position follow the same ECE sequence only at acidic media. At basic media, the mechanism consisted of a DISP1 scheme in which r.d.s. is the uptake of the proton in the N1 position by the OH^- ion of the media. In aprotic media the sequence was ECEC with the second step as the r.d.s.

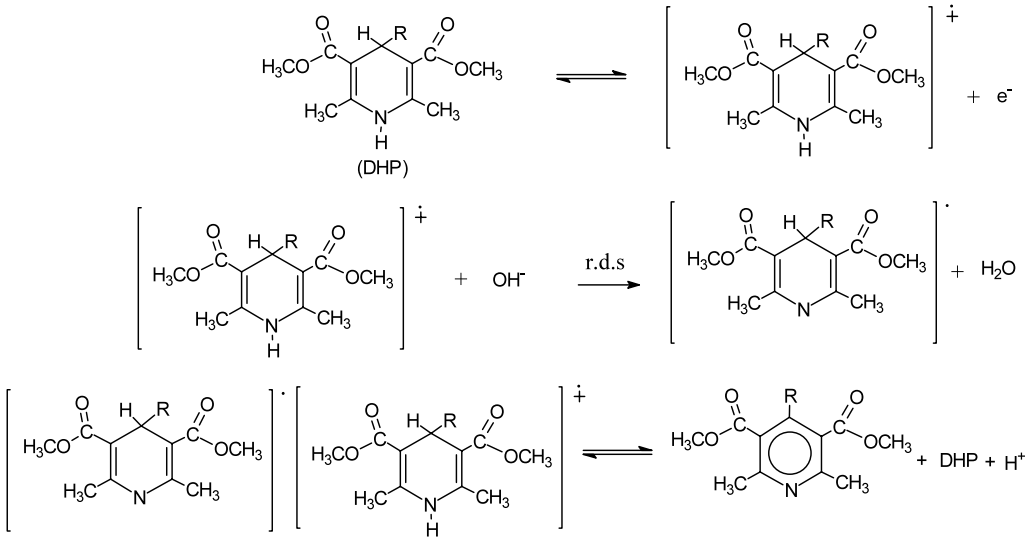
In aprotic and acid media compounds that have an ethyl-group instead of the H substituent in N1 position are easily oxidized due to the electron donating effect of the ethyl group. Moreover, in spite of in basic media both series of compounds follow different mechanisms, the presence of H substituent in N1 position produces easily oxidizable compounds.

In spite of in basic media both series of compounds follow different mechanisms, the presence of H substituent in N1 position produces easily oxidizables compounds portending that the proton on the amino secondary group of the 1,4-DHP play an important role in the facilitation of the oxidation process.

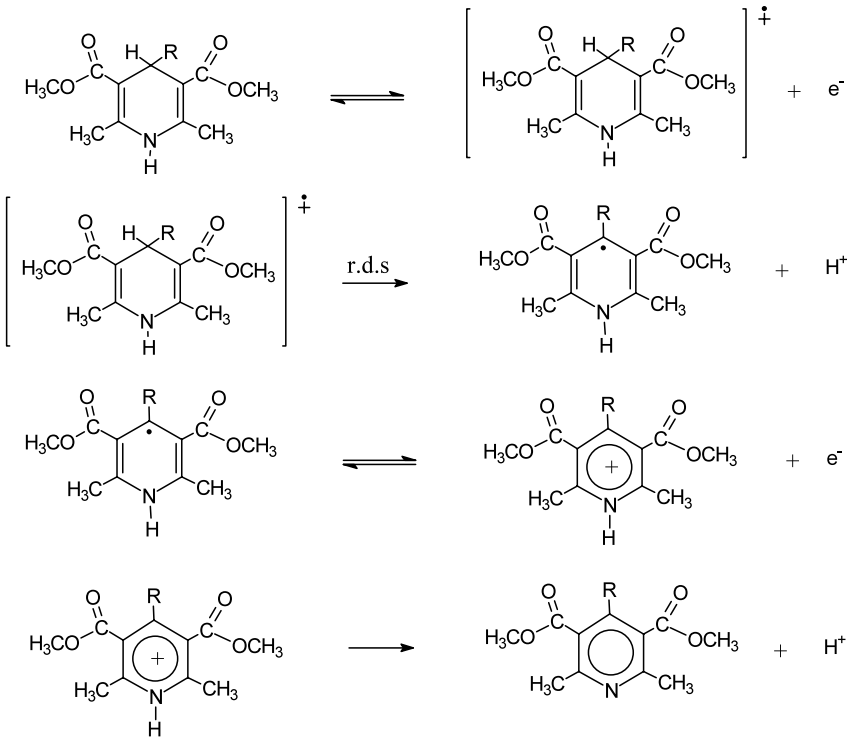
Using voltammetric, UV–Vis spectrophotometric and NMR spectroscopic evidences we have found that the hydrogen atom at the N1 position can react with a base as potassium superoxide producing the corresponding anion dihydropyridine which is easier oxidized. This behavior could be the key to explain the observed reactivity between 1,4-DHP and bases such as superoxide or nitric oxide recently informed [15,17]. This fact also qualify this type of 1,4-DHP as potential antioxidants or scavenger compounds.



Scheme 2. Oxidation mechanism of 1,4-DHP derivatives (**I**–**III**) in acidic media (pH < 4).



Scheme 3. Oxidation mechanism of 1,4-DHP derivatives (**I**–**III**) in basic media (pH >8).



Scheme 4. Oxidation mechanism of 1,4-DHP derivatives (**I**–**III**) in aprotic media.

Acknowledgements

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