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Investigation of light-induced free radicals in nifedipine

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Electron Paramagnetic Resonance (EPR) spectroscopy was used to study the behavior of the light-induced free radicals in the nifedipine [$C_{17}H_{18}N_2O_6$, 3,5-pyridine dicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitro-phenyl)- dimethyl ester], and to characterize the specific features of these radicals. Powder samples of nifedipine were investigated for a comparison between radiation damage in drug and in active substance. The nifedipine has not EPR signal before illumination or before UV irradiation, but the relative yielding of free radicals, appeared after irradiation, depends on the exposed time. By computer simulation of experimental spectra, some spectroscopic properties and suggestions concerning possible structure of the free radicals are discussed in this paper. Two radical species was found in pharmaceutical formulation tablets corresponding to active substance and ingredients.

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1. Introduction

It is well known that, the radiation on the drugs produces chemical and physical alterations and even to loss their biological activity [1]. This class of processes is included in photosensitization. The importance of the photosensitization processes can be understood taking into account the possible phototoxic effects induced by new pharmaceuticals which may be explained on the basis of the different biological effects induced by the photoproducts in relation to their parents molecules. The studies of photophysical and photochemical processes, including exam of excitation and emission properties, identification of reaction intermediates, isolation of photoproducts, analysis of interaction with biological substrates, are often an adequate approach to analyze the mechanisms through phototoxic effects can be produced. [2].

The photosensitiser could undergo a decomposition (most probably via homolytic process) so that the resulting photoproducts can act either as toxins or as new photosensitizes. One of illustrative example for these pathways may be found in the study of the reactivity of nifedipine which is a nitroaromatic compound used in the treatment of myocardial ischemia and hypertension.

This extreme photoinstability added to the fact that nifedipine is frequently prescribed for a prolonged therapy, was the reason to make some research for other nitroaromatics [3]

Electron Paramagnetic Spectroscopy (EPR) spectroscopy is a very useful method for the detection of the irradiated biological systems and now it plays a significant role in the characterization of free radicals obtained by illumination and by UV irradiation of drug and active form of nifedipine. When an unpaired electron in a magnetic field interacts with a nuclear spin, the spectrum splits into two or more lines, which produce hyperfine structure in the spectrum. The hyperfine structure of the EPR spectra, when it is well resolved, proved more important information about the free radicals, because most detected free radicals are nitrogen- and carbon-centered radicals and the spectra positions are almost in the same magnetic field.

The aim of the present work is to study by EPR spectroscopy the behavior of the illumination- and UV irradiation-induced free radicals in the nifedipine, to characterize the specific features of these radicals and stability of molecular compounds, on the exposed time.

2. Experimental

Pure nifedipine [$C_{17}H_{18}N_2O_6$, 3,5-pyridine dicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitro-phenyl)- dimethyl ester] purchase from Sigma-Aldrich and commercial pharmaceutical tablets, was exposed to the daylight and UV radiation at different exposed time, from 10 to 60 minutes. EPR spectra were recorded with an "ADANI Portable EPR Spectrometer PS8400", operating in the X-band (9.1GHz – 9.6GHz) equipped with a computer acquisition system. The spectrometer settings used for the experiments were as follows: modulation frequency, 100 kHz; modulation amplitude $2x10^3$; sweep width, 100 G; sweep time, 30 s; receiver gain $5x10^2$; number of data points, 4096. Using the same parameters, the spectra were recorded at different time intervals. The variation of the relative concentration of radical species, were obtained through double integration of the experimental spectra. For obtaining the magnetic characteristic parameters the experimental spectra was simulated using the program WINSIM02 [3].

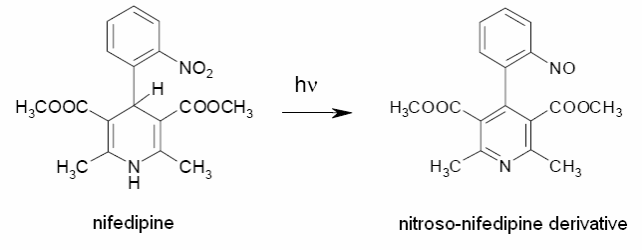

Fig. 1. Photo-degradation of nifedipine by UV and visible

3. Results and discussions

Nifedipine, an important drug used for the treatment of myocardial ischemia and hypertension, is extremely sensitive to ultraviolet radiation and to visible light up to 450 nm. Under daylight (natural) nifedipine give nitroso-phenylpyridine homologue, and under UV irradiation give the nitro-phenylpyridine homologue [4, 5] (Fig. 1).

The quantum yield for photodegradation is ~0.5; statistically this means that of every two photons absorbed, one causes decomposition of a nifedipine molecule.

By EPR spectroscopy were analyzed non-irradiated and irradiated samples of drug nifedipine. EPR measurements proved that, after irradiation, nifedipine converts mainly to 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester (NTP) which is a stable paramagnetic species [6]. The spin density of the unpaired electron in the NTP-adduct was centered on the nitrogen derived from its nitroso group. The relative yielding of the free radicals depends on the exposed time. No radical formation was observed for nonilluminated nifedipine. Also, the nifedipine yields a new nitroso spin trap which traps carbon-centered radicals and also forms relatively stable alkoxyl radical adducts (RO•) in triplet state. The triplet was attributed to intramolecular π-electron transfer [7]. By increasing the irradiation time the intensity of the EPR signal increased too, shown that the number of free radicals formed depending on the exposed time (Fig. 2 and Fig. 3).

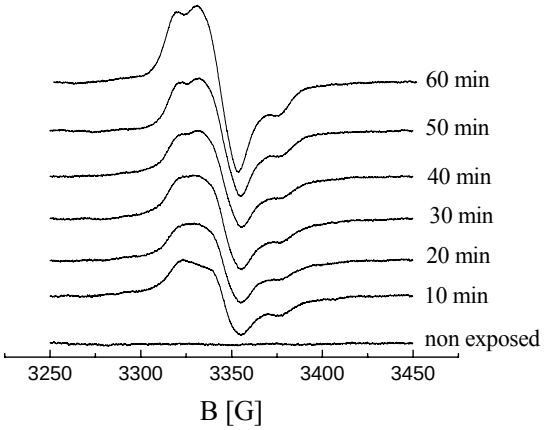

Fig. 2. X-band EPR spectra of dayligh exposed nifedipine.

The measurements for the UV irradiated nifedipine drug shown that, at higher energy the splitting of the spectrum is resolved better. It was observed a difference in the rate of increasing free radical species, because the intensity of the first line increases more quickly at UV light, than at visible light with a good resolved splitting as it is shown in Fig. 3.

Another difference is between drug nifedipine and pure nifedipine (Fig. 4). This difference is, probable, due to the potential of interactions between active pharmaceutical ingredients and the excipients used in pharmaceutical formulations. The presence of organic radical species in a commonly used excipient has been shown to accelerate the photodegradation of active pharmaceutical ingredients [7-9].

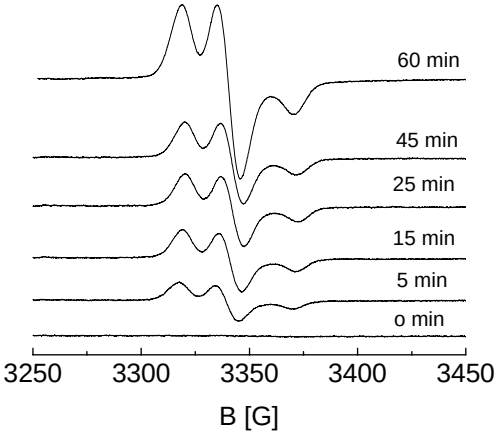

Fig. 3. X-band EPR spectra of UV irradiated nifedipine

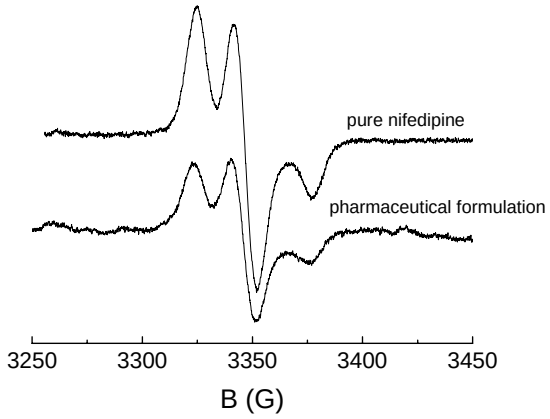

Fig. 4. EPR spectra of pure and pharmaceutical formulation nifedipine after UV radiations exposure.

By computer simulation of EPR spectra, some spectroscopic properties and suggestions concerning possible structure of the radicals are discussed. A good agreement between experimental and simulated spectrum was obtained assuming the existence of two radical species (Fig. 5). The first free radical is a radical centered on

nitrogen which gives a triplet with a line width of 7.39G (subspectrum 1). This species is due to the interaction of the unpaired electron with nitrogen, a(N)= 21.24G. The second free radical (subspectrum 2) gives a singlet with line width of 5.46G due to the interaction of unpaired electron with four protons: $a_1(H)$=5.95G and $a_2(H)$= $a_3(H)$= $a_4(H)$=10.26G centered on g=2.0035 and peak to peak line-width of 6.8 G. Compatible with these parameters can be a radical produced by breaking the bond between carbon and nitrogen from imidazolic group and addition of an hydrogen atom at one of the carbon atoms of the aromatic ring and thus, the unpaired electron occupies a highly delocalized orbital.

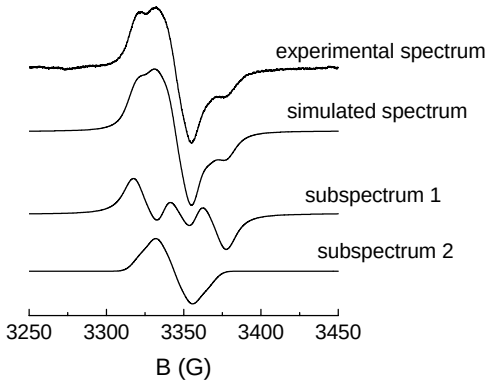

Fig.5. Experimental and simulated spectrum of illuminated nifedipine.

The effect of exposed time on radical yields in UV irradiated active substance of nifedipine is shown in Fig. 6.

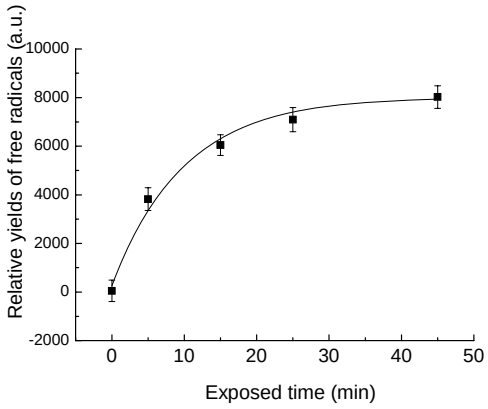

Fig. 6. The relative yields of radicals in UV irradiated pure nifedipine as function of exposed time.

The relative free radicals yields in the samples irradiated at each exposed time were determined by double integrated of corresponding EPR spectrum. The signal intensity, or the integral of the EPR absorption spectrum, is proportional to the number of spins in the sample, and can therefore be used as estimation for the relative concentration of the paramagnetic species. Experimental data were fitted by an exponential function, describing a first order kinetic of formation of the free radicals:

$$I(t) = I_{sat}\left[1 - \exp(-k \cdot t)\right]$$

in which I_{sat} is the limiting value corresponding to the steady state concentration of the radicals and k is the rate constant of destroying the radicals by the radiation

4. Conclusions

EPR spectroscopy may be used in order to analyze the effect of day- and UV light in pure and pharmaceutical formulated nifedipine. By computer simulation of experimental spectrum, we obtained the existence of two radical species generated by daylight and UV radiation. From the analysis of the EPR signal dependence on the different exposed times, we obtained that the rate of degradation, can be described with a first order kinetics pathway represented by exponential laws.

It can be concluded that nifedipine in a sensitive substance which decomposes in UV light to give the 4-(2-nitrophenyl) pyridine homologue, and under daylight to give the 4-(2-nitrosophenyl)-pyridine homologue with a kinetics of saturation at low value of exposed time (under 1 hour).

Acknowledgements

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