ELECTRON SPIN RESONANCE STUDIES OF γ-IRRADIATED B_3 VITAMIN

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Abstract. The aim of the present work is to study by ESR spectroscopy, the behavior of the γ-irradiation induced free radicals in the B_3 vitamin to characterize the species of these radicals and stability of molecular compounds, on the absorbed dose. The B_3 vitamin has not ESR signal before irradiation, but the relative yielding of free radicals depends on the absorbed dose. Some spectroscopic properties and suggestions concerning possible structure of the radicals are discussed in this paper.

Key words: Free radicals, gamma irradiation, ESR spectroscopy, vitamin B_3.

INTRODUCTION

The use of ionizing radiations is one of the most promising methods for the sterilization of solid pharmaceuticals. However the radiosterilization produces new products which can induce a modification of their chemical structure and to loss the biological activity [8]. Thus, stress testing of by γ-irradiation in drug substances can give information about the degradation pathways and the intrinsic stability of the molecule. During irradiation of solid drugs, free radicals are formed and trapped in the matrix. The fact that in free radicals, the unpaired electron is involved, these species are paramagnetic, the most used method for detecting free radicals is electron spin resonance spectroscopy (ESR) [5]. When an unpaired electron in a magnetic field interacts with a nuclear spin, the spectrum splits into two or more lines, which produce a hyperfine structure in the spectrum. The splitting of the spectrum is expressed in terms of a hyperfine coupling constant (*a* value in G or mT units), and the relative position of the spectrum is expressed by the spectroscopic splitting factor (*g* value, dimensionless). The ESR signal is detected only after irradiation and a part of free radicals produced survives at room temperature for a long time. From the decay curve of the radicals it is possible to estimate the initial dose of irradiation.

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In this paper, the kinetics of free radicals induced by γ-radiation in B_3 vitamin have been investigated by electron spin resonance (ESR) spectroscopy, to explore the possibility of using this technique in study of stability and damage in the microcrystalline powder form.

B_3 vitamin, also called nicotinic acid, is involved in a wide range of biological processes, including the production of energy, the synthesis of fatty acids, cholesterol and steroids, signal transduction, the regulation of gene expression and the maintenance of genomic integrity. Nicotinic acid given in drug dosage improves the blood cholesterol profile, and has been used to clear the body of organic poisons, such as certain insecticides. Also, nicotinic acid is used as radiosensibilizers incorporated in the radiant treatment and chemotherapy in carcenogenesis [7].

MATERIALS AND METHODS

Nicotinic acid ($C_6H_5NO_2$), with the chemical structure presented in Fig.1, was γ-irradiated using ^{60}Co source (GAMMA CHAMBER 900) which gives a uniform and compact density of radiation and a dose debit of 10.7 Gy/h.

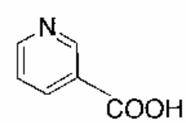

Fig.1. Structural formula of nicotinic acid.

Powder samples of B_3 vitamin irradiated at different absorbed doses (from 0 to 25 kGy) were used to generate the dose-response curve for the radicals associated with the integral of the ESR absorption spectrum. Samples irradiated at different doses were used to generate the dose-response curve for the radicals, irradiated at doses of 0.5, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0 and 25.0 kGy. ESR spectra were recorded with an "ADANI Portable ESR Spectrometer PS8400", operating in the X-band (9.1 GHz – 9.6 GHz) equipped with a computer acquisition system.

The computer simulation analysis of the spectra was made by using a program that is available to the public through the Internet for obtaining the magnetic characteristic parameters [6].

RESULTS AND DISCUSSIONS

ESR spectroscopy was used to analyse non-irradiated and irradiated samples of B_3 vitamin. By increasing the irradiation dose the intensity of the ESR signal increased too, shown that the number of radicals formed depending on the absorbed dose. The ESR spectra obtained by measurements consist of a broad central signal which is characteristic for free radicals in solid state.

ESR measurements proved that nicotinic acid contained various stable paramagnetic species after irradiation and relative yielding of the free radicals depends on the adsorbed dose (Fig. 2).

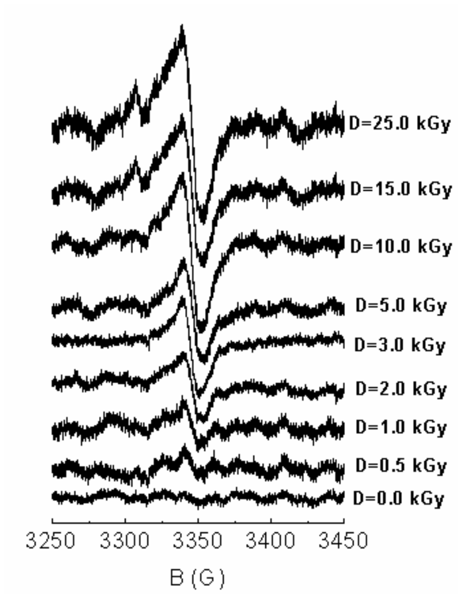

Fig.2. ESR spectra of γ-irradiated B_3 vitamin at different absorbed dose.

The total spectrum represents a sum of spectra corresponding to all free radicals simultaneously present in the sample, dominated by the broad central signal. These signals, exhibit a line width of 15.0 G, which reflect the random orientations of the radicals with regard to the magnetic and a broadening mechanisms contribution by dipolar and spin-spin interactions. The value of the isotropic *g*-factor of 2.005 is characteristic for carbon-centered radicals [1]. By computer simulation of ESR spectra, some spectroscopic properties and suggestions concerning possible structure of the radicals can be emphasize (Fig. 3).

A good agreement between experimental and simulated spectrum was obtained adopting the existence of two radical species. The first free radical, supposed to be a radical of type $R-\underset{\bullet}{C}OO^{-}$, formed by breaking a bond between carbon and hydroxyl group. The presents of $OH^{\bullet}$ radical can be explained by the fact that B_3 vitamin has hygroscopic characteristics and can be influence by the atmospheric water molecules. The hydrogen abstraction is the most common mechanism of producing free radicals in compounds containing aromatic rings [2]. This radical gives a doublet with 9.54 G peak-to-peak line width and is due to a proton with hyperfine coupling constant *a*(H) = 5.31 G.

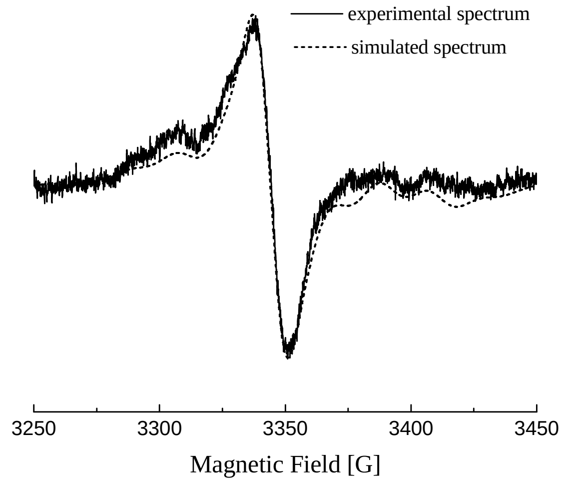

Fig. 3. Experimental and simulated spectrum of γ-irradiated B_3 vitamin at 25kGy absorbed dose.

The second free radical perhaps is the unpaired electron localized on nitrogen atom giving rise a characteristic hyperfine splitting with *a*(N) = 40.1 G. But also the unpaired electron interact with tree equivalent protons with hyperfine coupling a_1(H) = a_2(H) = a_3(H) = 17.2 G. In this case the peak-to-peak line width is 8.74 G.

The concentration of radicals (proportionally with double integrate of ESR signal normalized on milligrams of sample) grows exponentially at first and then at a progressively lower rate until a steady-state plateau is reached (Fig. 4). Plateau concentrations of radicals are very high which excludes the possibility of saturating all the traps. It appears that, during the irradiation, radicals are removed by rapid processes other than those which occur in the absence of radiation (after radiolysis).

The relative integral of the ESR absorption spectra intensities, i.e. dose-response curves, was obtained by best least-squares fit to the parameters, given by the following relative relationships:

$$I(D) = 4315 \cdot [1 - 1.02 \cdot \exp(-0.0001 \cdot D)] \tag{1}$$

This relation is similar to the expression, which describe the first order kinetics of radicals formation [3, 4]:

$$I(D) = I_{\text{sat}}\left[1 - \exp\left(-k \cdot D\right)\right] \tag{2}$$

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

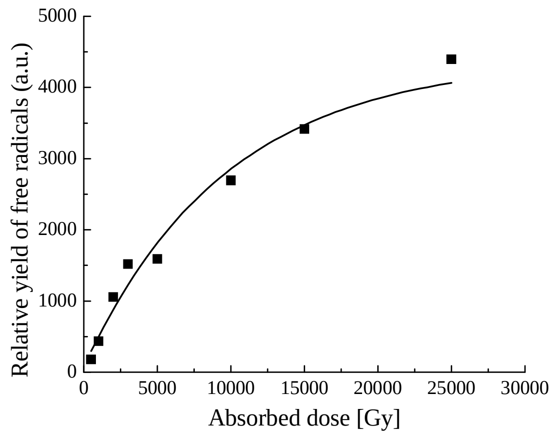

Fig. 4. The relative yields of radicals in irradiated B_3 vitamin as function of absorbed dose.

We can conclude that the B_3 vitamin has a high stability, because the kinetics saturation is at high value of absorbed dose.

CONCLUSIONS

From the analysis of the ESR signal dependence on the absorbed dose, it can be concluded that, γ-irradiation causes an increase in the amount of radicals represented by exponential laws with different parameters of generation and destroying.

Dose-response curve could be an informative tool for the study of the formation and recombination of the free radicals generated by high-energy ionizing radiation.

The high plateau concentrations of radicals generation can be attributed to the high stability of vitamin B_3 vs. ionizing radiation.

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REFERENCES

1. COZAR, O., V. CHIS, L. DAVID, G. DAMIAN, I. BARBUR, ESR investigation of gamma-irradiated aspirin, J. Radioanal. Nucl. Chem., 1997, 220(2), 241–244.

2. CRUCQ, A-S., Etude de mécanismes radicalaires de médicaments irradiés. Chim. Nouv. 1994, 12, 1356–1359.
3. DAMIAN, G., EPR investigation of γ-irradiated anti-emetic drugs, Talanta, 2003, 60, 923–927.
4. GIBELLA, M., A-S. CRUCQ, B. TILQUIN, P. STOCKER, G. LESGARDS, J. RAFFI, Electron spin resonance studies of some irradiated Pharmaceuticals, Radiation Physics and Chemistry, 2000, 58, 69–76.
5. GORDY, W., Theory and Applications of Electronic Spin Resonance, Ed. John Wiley, New York, 1980.
6. http://epr.niehs.nih.gov.
7. KIRKLAND, J.B., Niacin and Carcinogenesis, Nutrition and Cancer, 2003, 46(2), 110–118.
8. RAFFI, J., S. GELLY, L. BARRAL, F. BURGER, P. PICCELLE, P. PRINDERRE, M. BARON, A. CHAMAYOU, Electron paramagnetic resonance of radicals induced in drugs and excipients by radiation or mechanical treatments, Spectrochimica Acta part A, 2002, 58, 1313–1320.