STUDY OF FREE RADICALS IN GAMMA-IRRADIATED METOCLOPRAMID USING SPIN TRAPPING ESR SPECTROSCOPY

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Abstract. Spin trapping Electron Spin Resonance (ESR) spectroscopy was used to investigate the free radicals in γ-irradiated microcristalline powder form of 4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxy benzamide monohydrochloride monohydrate (metoclopramide). ESR measurements proved that contained various stable paramagnetic species after irradiation and relative yielding of the free radicals depends on the adsorbed dose. Specific radicals derived from purely chemical structures of metroclopramide, were detected using N-t-Butyl- α-phenylnitrone (PBN) as spin trap. Some spectroscopic properties and suggestions concerning possible structure of the radicals are discussed in this paper.

Key words: metoclopramide, ESR spectroscopy, spin trap, PBN.

INTRODUCTION

Free radicals are chemical species that possess an unpaired electron in the outer shell of the molecule. For irradiated drugs, they can be generated by homolytic cleavage of a covalent bond, in which a normal molecule fragments into two, each fragment retaining one of the paired electrons. This mechanism occurs, mainly in case of radiosterilization processes. During irradiation of solid drugs, free radicals are formed and trapped in spur [4, 12, 13, 16, 15]. Beside the specific radicals derived from purely chemical structures, in some drugs, like metroclopramide, hydroxyl radicals are generated. The fact that in free radicals, the unpaired electron is involved, these species are paramagnetic, thus the most used method for detecting free radicals is electron paramagnetic resonance spectroscopy (EPR).

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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). There exist two possibilities to use EPR spectroscopy in the detection of free radicals, depending on the their mobility and on the phase of the system in which they are generated. In case of solid systems, free radicals can be detected directly due to the low capacity to combine [7, 10]. In the liquid or gas phase two several problems arise when considering measurement of free radicals: First, the ultra-short half-life of these radicals (usually measured in microseconds). Second, any free radicals produced in vivo react at or close to their source of formation. Therefore, it is necessary to use a diamagnetic reagent named "spin trap" and to produce a relatively persistent product radical "spin adduct" which can be studied by conventional EPR (indirect detection) [1, 2, 10]. The intensity of the spin adduct EPR signal corresponds to the amount of short-lived radicals trapped, and the hyperfine splittings of the spin adduct are generally characteristic of the original, short-lived, trapped radical. A third problem is that many of these end products are in themselves reactive although to a lesser degree. Free radicals attack aromatic compounds and therefore the nitrones can be used to react with transient radicals to form longer-lived nitroxides (spin trapping). The nitrone spin trap is widely used to provide evidence for the involvement of free radicals in many biological and chemical reactions.

One of the most studied free radical species is hydroxyl radical. The hydroxyl radical is an extremely reactive oxidizing radical that will react to most biomolecules at diffusion controlled rates, which means that reactions will occur immediately with biomolecules [5]. The hydroxyl free radical is important in radiobiological damage and is several orders of magnitude more reactive towards cellular constituents than superoxide radicals (and many orders more reactive than hydrogen peroxide). In this paper we propose a method to detect such very reactive radicals, using Electron Paramagnetic Resonance spectroscopy (EPR).

MATERIALS AND METHODS

Metoclopramide (4-amino-5-chloro-N-[(diethylamino)ethyl]-2-methoxy benzamide mono-hydrochloride monohydrate) (Fig. 1) was purchased from Medicine Research Center, Beijing Shuangziao Pharmaceutical Corporation, China. Fresh metoclopramide in the form of microcrystalline powder was exposed to γ-radiation from a ^{60}Co source (GAMMA CHAMBER 900) in ambient conditions. The ^{60}Co source gives a compact and uniform density of radiations and a moderate dose debit of 35 Gy/h evaluated by ferrous sulfate dosimetry [6]. The absorbed dose of drugs was in the range from 0 to 17 kGy. Powder samples (non-irradiated, and irradiated) were placed in a 20 mm length, 1 mm inside diameter

quartz capillary. The mixture of irradiated metoclopramide and spin trap N-t-Butyl-α-phenylnitrone (PBN) was solved in acetone.

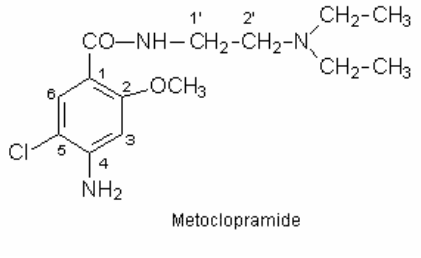


Fig. 1. Chemical structure of metroclpramide.

EPR spectra were recorded with "ADANI Portable EPR 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 WINSIM program that is available to the public through the internet [11].

RESULTS AND DISCUSSIONS

The EPR spectrum of γ-irradiated metoclopramide (Fig. 2) in solid state represent a sum of individual spectra corresponding to all free radicals simultaneously present in the samples or the same free radicals localized in various local environments.

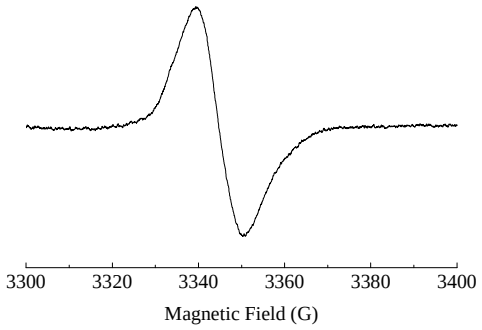
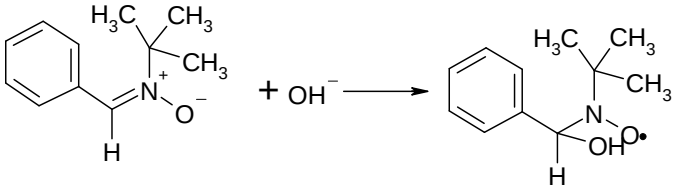


Fig. 2. ESR spectrum of irradiated metoclopramide.

The spectrum samples is dominated by a broad central signal with specific characteristics given by chemical structures, centered on g = 2.0047 an peak-to-peak line widths of 11 G. The values of the g-factor are characteristic for carbon- or nitrogen-centered radicals Due to the large values of line-widths it is very difficult to obtain the g and A parameters from the experimental spectrum. Therefore, the magnetic parameters corresponding to each radical were obtained by simulation of the spectrum. As shown in Fig. 3 there is a good agreement between experimental and simulated spectrum, was obtained by simulation with three radicals species. The first radical species generated in the irradiated metroclopramide, with hyperfine coupling constants A_1(H) = 3.8 G, A_2(H) = 3.0 seems to be a radical of type $R-\dot{C}OO^-$, formed by breaking chemical bond between amidic carbon and amidic nitrogen in the presence of some hydroxyl radicals from irradiated water molecules.

The presence of $OH^\bullet$ radical was not properly observed by classical methods, but their presence is motivated by the fact that metoclopramide is monohydrated and have hygroscopic characteristics.

To detect $OH^\bullet$ radical (named species 2 in Fig. 3) by the spin trapping method, we use acetone as neutral environment, which prevent the oxidation processes. The nitrones used as spin traps, N-t-Butyl- α-phenylnitrone (PBN) is a stable compound and forms relatively long-lived spin adducts with various types of radicals as in Scheme 1.



Scheme 1. Formation of spin adduct with PBN.

The characteristic features of this species having A(N) = 14.6 G, and A(H) = 3.1 G, are typical PBN/OH spin adduct [3]. During evaporation of acetone, the EPR spectra reassemble more to a spectrum of stable nitroxide radicals.

The hyperfine structure of the third radical of 5.8 G (species 3), centered on g = 2.0035 and peak to peak line-width of 3.7 G, is compatible with the 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 [8]. Similar addition processes have been observed as the result of radiation damage in other unsaturated organic compounds [9].

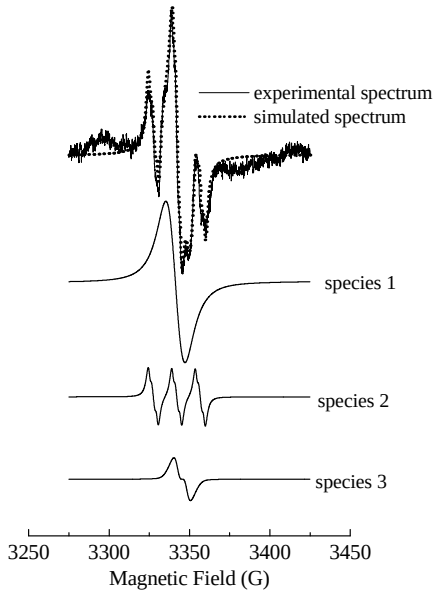


Fig. 3. Experimental and simulated ESR spectrum of PBN/metroclopramide in acetome.

CONCLUSIONS

The free radicals generated in the solid drugs in different stress conditions, can be detected by the spin trapping method using adequate solvents to obtain specific spin adduct, detectable by EPR spectroscopy.

The obtained spin adducts have specific features expressed by different living-times. Thus, during the EPR measurements, we observed a time dependence of the spectral characteristics and signal intensities.

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