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ESR detection and dosimetric properties of irradiated naproxen sodium

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Abstract

In the present work, the electron spin resonance (ESR) dosimetric properties of naproxen sodium (NS) was investigated in the dose range of (2.5–25 kGy). Irradiated NS exhibited a very simple ESR spectrum consisting of a broadened antisymmetric single resonance line not saturating up to 2 mW microwave power at room temperature. The sum of two exponential increasing functions associated with two different radicals of different spectroscopic features and relative weights were found best describing experimental dose–response curve. Radiation induced radicals were observed to be very stable at room temperature but the increase in storage temperature increased very appreciably the decay of the contributing radicals. The results of the simulation calculation based on a model of two radicals showed that two carbon dioxide ionic free radicals $(^{\bullet}CO_2)^-$ of different orientational and environmental features produced by preferential rupture of carboxyl group from the rest of NS molecules in the crystalline matrix, were, likely at the origin of the experimentally observed ESR spectrum. Features such as good time stability of the signal intensity and relatively high radiation yield ($G = 0.13$) were considered providing NS with potential use as dosimetric material in measuring radiation dose in the range of 2.5–25 kGy by ESR technique.

Keywords: ESR; Naproxen sodium; Irradiation; Radicals

1. Introduction

Radiation sterilization technology and its applications in the manufacture of pharmaceuticals and cosmetics are being more actively investigated now than at any other time (Jacobs, 1995; Reid, 1995; Tilquin and Rollmann, 1996; Boess and Bögl, 1996). The increased use of radiation processing for other industrial purposes has led to the development of more efficient and economical irradiation equipment and processes. On the other hand, due to improvements made in aseptic processing, it is possible to obtain products and materials much cleaner from a microbiological point of view and thus are likely to require much lower radiation doses to achieve 10^{-6} sterility assurence level (SAL). This progress provides an opportunity to terminally sterilize a large number of drugs by radiation. However, ionizing radiation produces degradation products (Schuttler and Bögl, 1993; Miyazaki et al., 1994) which may result in a toxicological hazard. To prove the safety of radiosterilization, it is important to determine and quantify the radiolytic degradation and elucidate the mechanism. Electron spin resonance (ESR) appears to be well suited for this purpose. In fact, the species and amounts of the molecular fragments or radicals possessing unpaired electron

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created by radiation can be best detected by ESR spectroscopy which is frequently used to measure the absorbed dose of an irradiated material (Dood et al., 1985; Desrosiers and Simic, 1988; Bögl, 1989; Raffi, 1992; Onori et al., 1996; Polat et al., 1997; Basly et al., 1998; Korkmaz and Polat, 2000,2001) and hence, distinguish it from unirradiated ones.

The purpose of the present work is to apply the ESR spectroscopy to the irradiation dosimetry of NS and consequently, to investigate the radiation sensitivity of this drug in the dose range of 2.5–25 kGy by developping best mathematical equations describing the variation of ESR intensities versus dose and storage time after irradiation and to perform simulation calculations to determine and characterize radiolytical products contributing to the experimental ESR spectra of irradiated NS.

2. Materials and methods

Naproxen sodium (NS), its chemical name is 2-naphthaleneacetic acid, 6-methoxy-α-methyl sodium salt ($C_{14}H_{13}NaO_3$), was provided from Abdi Ibrahim Drug Company (Istanbul). NS is an odorless, crystalline powder, white to creamy in color and has a molecular structure as given in Fig. 1. It is soluble in methanol and water. The NS samples were irradiated at room temperature (290 K) in the dose range of 2.5–25 kGy using a ^{60}Co gamma cell supplying a dose rate of 2.5 kGy/h as an ionizing radiation source at the Sarayköy Establishment of Turkish Atomic Energy Agency in Ankara. The dose rate at the sample site was measured by a Fricke dosimeter.

ESR measurements were carried out using a Varian 9″ E-L X-band ESR spectrometer operating at 9.5 GHz and equipped with a TE_{104} rectangular double cavity containing a DPPH standard sample in the rear resonator which remained untouched throughout the experiment. The spectrometer operating conditions adopted during the experiment are given in Table 1. Signal intensities were calculated from the first derivative spectra and compared with that obtained for a standard DPPH sample under the same spectrometer operating conditions.

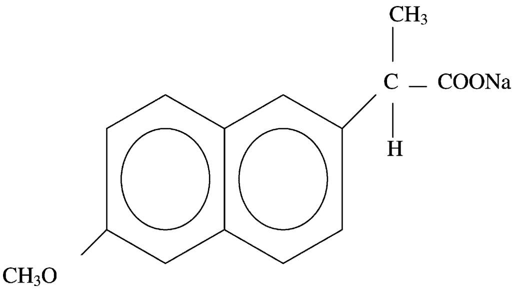


Fig. 1. Molecular structure of naproxen sodium.

Table 1
ESR spectrometer operating conditions adopted throughout the experiment

Central field	329 mT
Sweep width	20 mT
Microwave frequency	9.26 GHz
Microwave power	1 mW
Modulation frequency	100 kHz
Modulation amplitude	0.5 mT
Receiver gain	5×10^3
Scan time	240 s
Time constant	1 s

3. Experimental results and discussion

3.1. ESR spectra of irradiated NS

Although unirradiated NS exhibited no ESR signal, irradiated NS showed a very simple ESR spectrum consisting of a broadened antisymmetric single resonance line centered at $g = 2.0057 \pm 0.0008$ as shown in Fig. 2a and b, respectively. Room temperature microwave saturation features of this signal were studied first in the range of 0–50 mW and the behavior of single radical species was observed. No saturation was observed up to 2 mW microwave power at room temperature and a microwave power of 1 mW was adopted throughout the experiment in the present work.

3.2. Dose–response curve

Variation of peak-to-peak signal intensity versus to absorbed dose in the applied dose range of 2.5–25 kGy was given in Fig. 3. The concentration of radicals in irradiated NS, grows linearly at the beginning and then at a progressively lower rate until a relatively steady plateau is reached in the studied dose range. It appears that during irradiation radicals

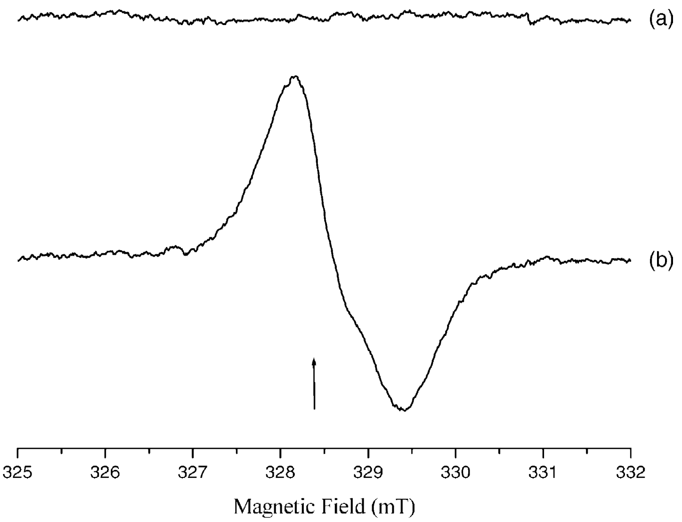


Fig. 2. ESR spectra of naproxen sodium. (a) unirradiated (control) and (b) irradiated at 15 kGy. Arrow indicates the position of DPPH resonance line.

are removed by relatively rapid processes other than those which occur after cessation of irradiation. Simulation calculations given in Section 3.4 showed that two different radicals (A and B) were produced by gamma irradiation in NS. So, these radicals was taken into consideration for developing a mathematical equation describing the dose–response curve. The sum of two exponential functions associated with radicals A and B were adopted for fitting the experimental signal intensity data. The best

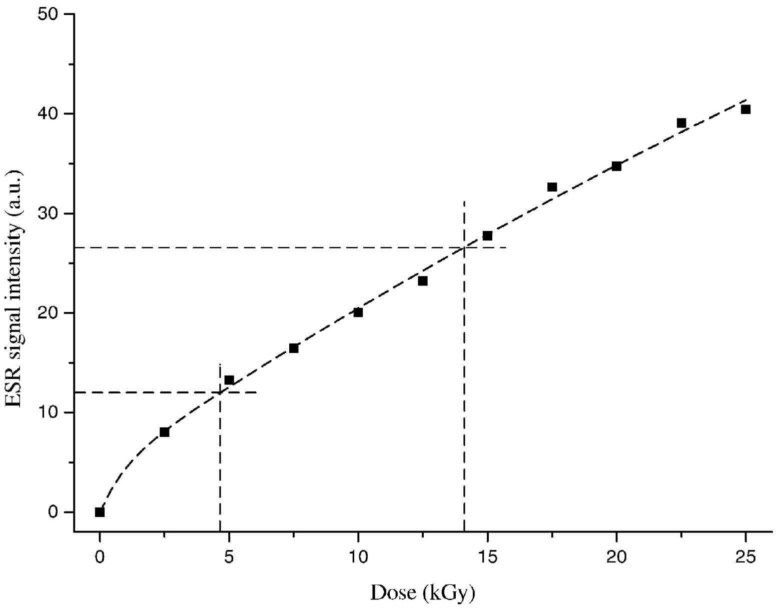


Fig. 3. Variation of ESR peak-to-peak intensity with absorbed radiation dose; (■) experimental, (---) calculated.

mathematical equation describing the ESR dose–response curve with a correlation coefficient of 0.9971 was found to be:

$$\text{ESR signal intensity} = 136.6 \times \left[1 - e^{-0.012 \times D}\right] + 4.17 \times \left[1 - e^{-1.025 \times D}\right]$$

In this equation, D stands for the applied radiation dose in kGy. The first term in the equation is due to radical A and the second term is due to radical B. The relative weights of the radical A and B at the applied dose of 15 kGy calculated from the above equation were 23.69 and 4.17, respectively. These results are in agreement with the results calculated from simulation calculations given in Section 3.4. The theoretical dose–response curve calculated from the above equation and its experimental counterpart are both given in Fig. 3. The theoretical dose–response curve was used to determine radiation dose received two samples irradiated at 5 and 15 kGy. The signal intensity values 12.0±1.0 and 26.5±1.5 obtained from their ESR spectra were used to determine the intersect points on the theoretical dose–response curve (Fig. 3). The corresponding radiation doses were calculated by sketching vertical lines from the intercept points to the dose axis. The calculated doses by this technique for the samples irradiated actually at 5 and 15 kGy were determined to be 4.6 ± 0.5 and 14.1 ± 1.4 kGy, respectively. The doses received by these samples was also calculated by replacement of the ESR signal intensity values in the theoretical dose–response curve function and solving the equation for D. The received doses found by this procedure were as 4.7±0.5 and 14.0±1.3 kGy for the samples irradiated at 5 and 15 kGy, respectively.

3.3. *The effects of storage time and temperature on radical kinetics*

NS samples irradiated at different radiation doses (5, 10, 15 and 20 kGy) were stored at room temperature (290 K) for 10 weeks. The ESR spectra of the samples were recorded separately, at the end of each week. Variations of the peak-to-peak signal intensities with storage time for samples irradiated at different radiation doses were given in Fig. 4. As can be seen from this figure, radiation-induced radicals in NS are quite stable at room temperature in the experimental error limits. The effect of storage temperature on the radiation-induced radicals was also investigated. For this purpose, five samples irradiated at 10 kGy were stored at different temperature (308, 323, 338, 358 and 373 K) for 1.5 h. Then, their ESR spectra was recorded at room temperature (290 K). The decrease

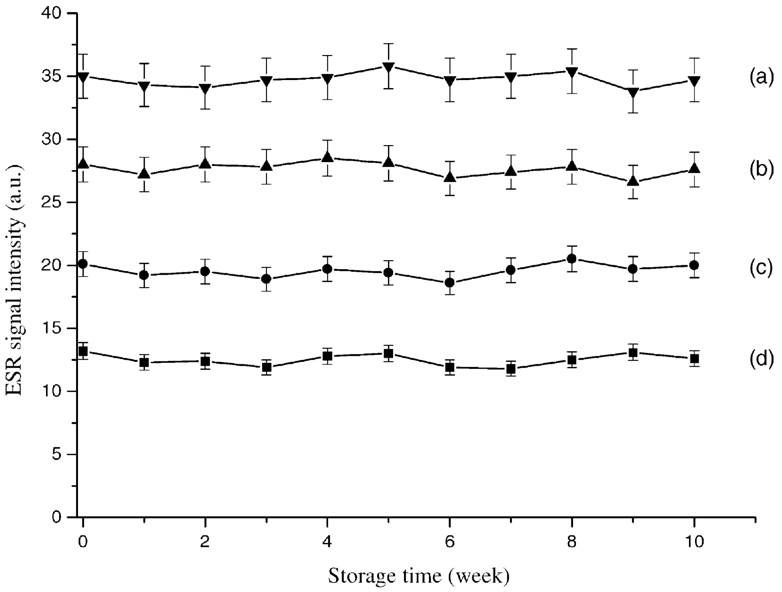
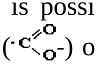


Fig. 4. Variation of ESR peak-to-peak intensity with storage time. Sample irradiated at (a) 20, (b) 15, (c) 10 and (d) 5 kGy.

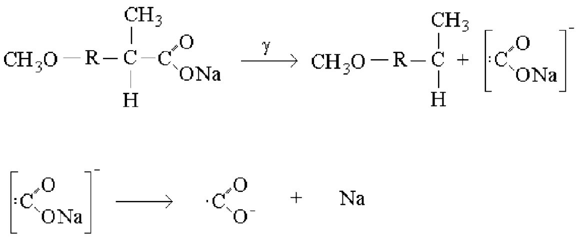
in the peak-to-peak signal intensity was calculated for each sample stored at different temperatures and the following values were obtained 6.2, 19.6, 28.6, 34.7 and 42.2% for the samples stored at 308, 323, 338, 358 and 373 K, respectively. This is an expected result, because high temperatures increase the motional freedom of the radiation-induced radicals which undergo destruction. These results indicate that radiation-induced radicals in NS are highly sensitive to the storage temperature.

3.4. Proposed radicals

Excited molecules are produced both directly and by neutralization of radical-cations (Dusaucy and Tilquin, 1991). They may decompose to radicals by rupture of chemical bonds. However, these radicals which are molecular fragments, do not diffuse in solid matrices due to cage effect and consequently immediate germinate termination reactions are possible (Tilquin, 1985). Excited molecules and radical cations are localized along the track in region of high local concentration. So the radicals are also formed in spurs and kinetic must take the effect of non homogeneous formation of the species into consideration.

It is difficult to presume, a priori, the bond that would be ruptured by gamma irradiation, because, NS contains many kinds of chemical bonds. However, independence of the ESR signal shape from applied microwave power and received radiation dose and the lack of hyperfine structure indicate that gamma radiation induces, in NS, radical species of similar spectroscopic features and that hydrogen atoms do not exist in the vicinity of the unpaired electron of these radicals in the dose range of 2.5–25 kGy. The broad antisymmetric single line is possibly due to carbon dioxide ionic free radical ( $\cdot C(=O)O^-$) of different orientational and environmental features produced by preferential rupture of carboxyl group from the rest of NS molecules in the crystalline matrix. In fact, naproxen which is expected to have similar crystal structure with NS, crystallizes in monoclinic system with $P2_1$ symmetry and contains two molecules per unit cell (Ravikumar et al., 1985). Carbon dioxide ionic free radicals giving rise to broad single resonance line have been also observed in irradiated HCOONa (Overnall and Whiffen, 1961), $KHCO_3$ (Chantry et al., 1962) and natural calcite (Marshall and McMillan, 1968).

Two different radicals of similar orthorombic g tensor but having different orientational and environmental characteristics were considered for simulation of room temperature spectra. The possible production mechanism of such carbon dioxide ionic radicals would be as given below:



Simulation calculations were performed using a mathematical model based on the sum of Gaussian shape functions associated with the carbon dioxide radicals assigned as A and B. The anisotropy originating from the tetragonal symmetry of g factor was also included in the calculations. Table 2 summarizes the calculated parameter values best fitting the experimental room temperature spectra. Theoretical ESR spectrum derived using the parameter values given in Table 2 and corresponding room temperature experimental spectrum are given, together, in Fig. 5 for gamma irradiated NS for comparison.

Table 2
Spectral parameter values calculated for proposed radical species A and B and the data given in the literature for CO_2^- ionic radical

Radical species	Material	Relative weight	$\Delta H_{1/2}$ (mT)	g tensor principal elements				Reference
				g_{xx}	g_{yy}	g_{zz}	g_{av}	
A	$C_{14}H_{13}O_3Na$	4.2	0.282	2.0037	1.9973	2.0010	2.0007	This work
B	$C_{14}H_{13}O_3Na$	25.6	0.538	2.0038	1.9978	2.0005	2.0007	This work
CO_2^-	HCOONa	–	–	2.0032	1.9975	2.0014	2.0007	Overnall and Whiffen (1961)
CO_2^--H	$KHCO_3$	–	–	2.0031	1.9971	2.0012	2.0005	Chantry et al. (1962)
CO_2^--F	Natural calcite	–	–	2.0035	1.9980	2.0022	2.0012	Marshall and McMillan (1968)

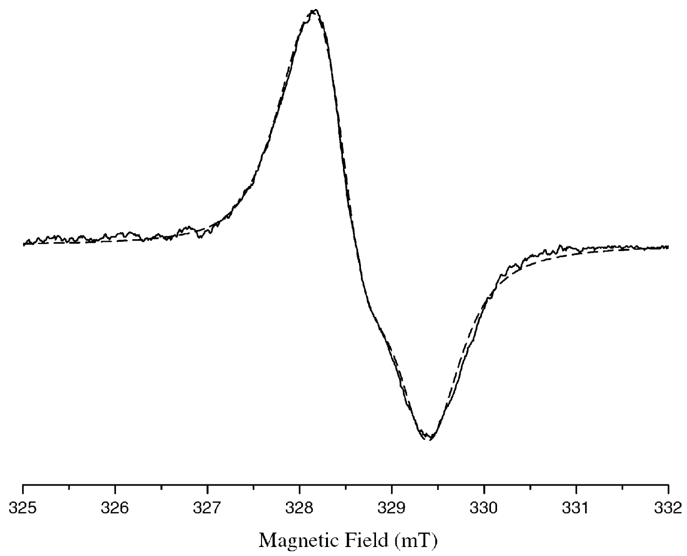


Fig. 5. Experimental and simulated room temperature spectra of naproxen sodium irradiated at 15 kGy. (—) experimental, (---) calculated.

4. Conclusion

The relative weights of the radicals A and B calculated from biexponential function given in Section 3.2 best fitting the experimental data are in good agreement with those derived from simulation calculations (Table 2). This indicates that radical A is produced much easily than radical B, although the other spectroscopic features of these radicals are quite similar. This difference in production rate may arise from the local environmental differences of the NS molecules in the crystalline lattice.

As is given in Table 2, calculated principal *g* values of the radicals A and B are quite close to those reported in the literature for carbon dioxide ionic radical (CO_2^-). Hence, it would not be wrong to conclude that carbon dioxide ionic radical are also produced in irradiated NS. However, although principal *g* values of the produced radicals are very similar, their orientations in the crystalline lattice of NS are quite different, so that they give rise to an antisymmetric single resonance line as shown in Fig. 2.

Gamma irradiation of NS produces free radicals which are detectable by ESR and appear to be relatively stable at room temperature. Two radicals assigned as A and B contribute to the ESR spectrum of observed for irradiated NS. Therefore, ESR measurements can be used for detection and discrimination of unirradiated NS from irradiated ones. The shape of decay curves over a storage period of 10 weeks at room temperature indicates that the free radicals could be detected even after a storage period of several months in irradiated NS.

The number of free radicals for each applied dose was estimated by comparison of the second integrals obtained from experimental spectrum of irradiated sample with that obtained for the DPPH standard. To get an integrated relative area, all spectra were corrected for baseline using a linear function and doubly integrated. The number of free radicals estimated by this procedure was $(8 \pm 2)\ 10^{15}$ spin/*g*/kGy. From this a *G* value of 0.13 was found. This value is 1 for alanine (Ikeya, 1993).

The ESR signal recorded for NS is specific for radiation treatment because no ESR signal was detected in unirradiated samples. Relatively big half times of two contributing radicals provide long time stability to the ESR signal at room temperature (Fig. 4). Therefore, ESR can be used safely to get both qualitative information (i.e. whether or not NS has been irradiated) and quantitative results (i.e. the dose it received) up to the recommended maximum radiosterilization dose which has been established by many regulatory authorities (EN 552, 1994) and (ISO 11137, 1995), because the contributing radicals are quite stable, the relative signal is clearly distinguishable from that of reference sample and the signal is relatively constant to estimate initial dose.

The results given above indicate that NS can be used as a dosimetric material. But, the sensitivity of the radiation-induced radicals to the storage temperature must be taken into consideration.

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