The effects of gamma irradiation on diclofenac sodium, liposome and niosome ingredients for rheumatoid arthritis

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Abstract: The use of gamma rays for the sterilization of pharmaceutical raw materials and dosage forms is an alternative method for sterilization. However, one of the major problems of the radiosterilization is the production of new radiolytic products during the irradiation process. Therefore, the principal problem in radiosterilization is to determine and to characterize these physical and chemical changes originating from high-energy radiation. Parenteral drug delivery systems were prepared and *in vitro* characterization, biodistribution and treatment studies were done in our previous studies. Drug delivery systems (liposomes, niosomes, lipogelosomes and niogelosomes) encapsulating diclofenac sodium (DFNa) were prepared for the treatment of rheumatoid arthritis (RA). This work complies information about the studies developed in order to find out if gamma radiation could be applied as a sterilization method to DFNa, and the raw materials as dimyristoyl phosphatidylcholine (DMPC), surfactant I [polyglyceryl-3-cethyl ether (SUR I)], dicethyl phosphate (DCP) and cholesterol (CHOL) that are used to prepare those systems. The raw materials were irradiated with different radiation doses (5, 10, 25 and 50 kGy) and physicochemical changes (organoleptic properties pH, UV and melting point), microbiological evaluation [sterility assurance level (SAL), sterility and pyrogen test] and electron spin resonance (ESR) characteristics were studied at normal (25 °C, 60% relative humidity) and accelerated (40 °C, 75% relative humidity) stability test conditions.

Keywords: diclofenac sodium, DMPC, SUR I, DCP, cholesterol, gamma irradiation, sterilization

Introduction

One of the most critical parameters for injectable controlled release drug delivery systems is sterility. Those systems have several advantages which make them preferable systems for the pharmaceutical industry [1]. In pharmaceutical industry, sterilization methods such as steam, dry heat, ethylene oxide gas, filtration and chemical processes have been used to obtain microbial reduction. Gamma irradiation has becoming an alternative sterilization method for the pharmaceutical industry, recently [2]. Also, it has turned out to be an interesting and promising technique for drug delivery systems like liposomes [3–4], which are defined as lipid bilayer vesicles that can encapsulate drugs either in aqueous compartment or inside of the lipid bilayer and transport them to the cells efficiently [5]. Nowadays, gamma irradiation is used to sterilize and/or decontaminate several pharmaceuticals, raw materials and finished products successfully [4–11]. Different sterilization techniques have been used and reviewed for liposomes by Zuidam et al. [12], however, there is still an unsolved problem for the irradiation process which causes radiolytic products [13]. The main reason for the oxidative damage is free radicals such as hydroxy radicals originating from exposure of water to radiation. Moreover, water content plays a key role in the stability of liposomes during the sterilization process [2]. As conclusion, the main approach for the radiosterilization process is to determine and characterize the physical and chemical changes which are the results of high energy radiation [4, 14–15].

In this study, the effects of gamma irradiation on DFNa and phospholipids and surfactants which are

used for the preparation of drug delivery systems are investigated in more detail. Physicochemical (organoleptic properties, pH, UV and melting point), analysis of DFNa is followed by ESR analysis and microbiological tests including determination of sterility assurance level (SAL) levels, sterility and pyrogen tests.

Materials and Methods

DFNa was kindly provided by Deva (İstanbul, Turkey). DMPC, SUR I and DCP were supplied by Phospholipid GmbH (Köln, Germany), L'Oreal (Paris, France) and Sigma (USA), respectively. All other chemicals were of analytical grade.

Irradiation procedure

All irradiations were performed under normal conditions (25 °C, 60% relative humidity) in dark using a ^{60}Co gamma cell (4523 Ci, Hungary) supplying a dose rate of 1.28 kGy·h^{-1} as an ionizing radiation source at the Sarayköy Gamma Irradiation Facility of Turkish Atomic Energy Agency in Ankara.

All investigations including (organoleptic properties, pH, UV and melting point) were performed on samples (DFNa, DMPC, SUR I, DCP, CHOL) irradiated at four different dose levels (5, 10, 25 and 50 kGy). Unirradiated samples were used as controls to detect physicochemical and antimicrobial activity changes resulting from the action of ionizing radiation on studied samples.

Organoleptic properties

Organoleptic properties (odor, appearance, clarity, color) of raw materials were performed before and after gamma irradiation.

pH measurements

pH measurements of the control (unirradiated) and irradiated raw materials were performed using pH-meter (WTW Inolab, Germany) before and after irradiation.

UV measurements

λ_{max} values of raw materials were measured in HEPES buffer (pH = 7.4) with UV spectrophotometer (Schimadzu UV 160A, Japan) before and after irradiation.

ESR measurements

ESR measurements of raw materials and DFNa-loaded formulations were carried out using Bruker EMX 113 spectrometer operating at 9.5 GHz. The spectrometer operating conditions adopted during the experiments are given in *Table I*.

The results of DMPC were the average of ten replicates for each radiation dose level. To ensure that power saturation did not occur, a microwave power of 1 mW was set during the experiments.

Sterility test

For the sterility test, two media were used [fluid thioglycolate medium (FTM) and tryptic soy broth (TDB)]. Raw materials were shaken in sterile distilled water and 100 µl of water was inoculated to FTM and TDB. They were incubated 14 days, at 35 °C and 25 °C, respectively. After 14 days, the tubes that are turbite were considered as non-sterile and the tubes which are clear were considered as sterile [16].

Table I ESR spectrometer operating conditions adopted throughout the experiments

Central field	350.0 mT
Sweep width	20 mT
Microwave frequency	9.85 GHz
Microwave power	1 mW
Modulation frequency	100 kHz
Modulation amplitude	0.1 mT
Receiver gain	6.3×10^3
Sweep time	83.89 s
Time constant	327.68 s
Conversion time	81.92 s
Temperature	RT (room temperature, 25 °C)

Pyrogen (LAL) test

The gel-clot method for assay of bacterial endotoxins (the most common pyrogens) was examined for the above-mentioned substances [16].

SAL determination

The samples were infected with *Bacillus pumilus* spore suspension [6×10^6 colony-forming unit (cfu·ml^{-1})] and irradiated with various radiation dose levels (1, 5, 10, 25 and 50 kGy) and incubated in TSB plates at 35–37 °C. *Bacillus pumilus* colonies were enumerated and cfu in 1 mL were calculated. SAL 10^{-6} dose was calculated from the logarithmic microorganism death graphics [17].

Stability tests

Stability tests were performed under normal (25 °C, 40% relative humidity) and accelerated (40 °C, 75% relative humidity) conditions over a period of 3 months. For the accelerated stability test, the samples were stored in the climated chamber and aliquots were taken off for the measurements. For comparison unirradiated samples were used as negative control.

Results and Discussion

This study describes investigation on ionizing radiation induced oxidative damage to DFNa and phospholipids/surfactants following gamma irradiation. Color change in the irradiated raw materials is a simple and helpful observation to get information about possible radiolytic intermediates produced in these substances upon irradiation. As no color change was observed in irradiated solid materials in the applied dose region of 5–50 kGy, it can be concluded that either radiolytic intermediates are not produced by irradiation in studied samples or created intermediates do not exhibit any absorption in the visible region. The negative result in color change of the present work is consistent with the results reported in the literature for similar compounds [18]. Experimental results showed that organoleptic features such as color, odor and clarity and odor of control and irradiated samples did not change under accelerated test conditions before and after irradiation ($p < 0.05$). The results were in accordance with the literature [19].

pH values of raw materials changed after irradiation at four different dose levels ($p > 0.05$). The results are given in *Table II*.

λ_{max} values of DFNa, DMPC, DCP, SUR I and CHOL are found as 275, 245, 271, 256 and 242 nm, respectively, before gamma irradiation. The comparative λ_{max} values determined from UV spectrum of samples are given in *Table III*. As seen from the table, λ_{max} values

Table II Measured pH values for control and irradiated samples ($n = 6$)

Substance	pH				
	Dose rate (kGy)				
	0	5	10	25	50
DFNa	7.44 ± 0.03	7.37 ± 0.01	7.34 ± 0.02	7.24 ± 0.02	7.12 ± 0.02
DMPC	7.41 ± 0.01	7.41 ± 0.06	7.38 ± 0.01	7.30 ± 0.01	7.22 ± 0.03
DCP	7.40 ± 0.05	7.35 ± 0.03	7.33 ± 0.03	7.27 ± 0.03	7.21 ± 0.03
SUR I	7.43 ± 0.02	7.40 ± 0.01	7.35 ± 0.01	7.31 ± 0.01	7.28 ± 0.03
CHOL	7.44 ± 0.01	7.41 ± 0.02	7.40 ± 0.01	7.34 ± 0.03	7.18 ± 0.04

Table III λ_{max} values calculated from UV spectrum of unirradiated and irradiated samples ($n = 6$)

Substance	λ_{max} (nm)				
	Dose rate (kGy)				
	0	5	10	25	50
DFNa	275 ± 0.1	274 ± 0.3	274 ± 0.6	275 ± 0.1	275 ± 0.2
DMPC	245 ± 0.4	241 ± 0.1	243 ± 0.2	245 ± 0.5	244 ± 0.2
DCP	271 ± 0.1	270 ± 0.5	270 ± 0.4	273 ± 0.3	272 ± 0.4
SUR I	256 ± 0.3	256 ± 0.3	251 ± 0.1	254 ± 0.3	255 ± 0.3
CHOL	242 ± 0.1	240 ± 0.6	241 ± 0.2	240 ± 0.4	242 ± 0.2

Table IV ▌ Melting point values for unirradiated and irradiated samples (n = 6)

Substance	Melting point (°C)				
	Dose rate (kGy)				
	0	5	10	25	50
DFNa	271 ± 0.03	270 ± 0.01	273 ± 0.02	271 ± 0.05	270 ± 0.03
DMPC	22 ± 0.01	24 ± 0.03	24 ± 0.02	24 ± 0.01	21 ± 0.02
DCP	171 ± 0.06	170 ± 0.05	171 ± 0.06	171 ± 0.06	171 ± 0.06
SUR I	50.90 ± 0.04	51 ± 0.03	50 ± 0.01	52 ± 0.01	51 ± 0.05
CHOL	148 ± 0.06	145 ± 0.02	141 ± 0.01	144 ± 0.03	141 ± 0.01

calculated from UV spectrum did not change after irradiation ($p > 0.05$). The results are in good agreement with the literature [20–24].

Melting point values of DFNa, DMPC, DCP, SUR I and CHOL are 271 °C, 22 °C, 171 °C, 50.9 °C and 148 °C, respectively. Results are given in *Table IV* and were compared with unirradiated results.

According to organoleptic examination, pH, λ_{max} and melting point analysis, no significant change was found for all samples after irradiation with different radiation dose rates ($p > 0.05$).

Unirradiated DFNa, DMPC, DCP, SUR I and CHOL exhibited no ESR signal but irradiated samples did. Room temperature spectra recorded at two different radiation doses of gamma irradiation (5 kGy and 50 kGy) are given in *Figs 1* and *2* with the assigned peak numbers. Irradiated DFNa showed a well ESR spectrum having high resolution with three resonance peaks [10]. Increase in the absorbed dose caused only more intense spectra without creating any change in pattern. Thus, it was concluded that irradiation dose was not an important parameter in the formation of the shape of the ESR spectrum of DFNa [25]. Variations of the ESR peak heights measured with respect to spectrum base line with the applied microwave power was studied in the range of 0.02–100 mW using a sample of DFNa, DMPC, DCP, SUR I and CHOL irradiated at the dose of 25 kGy. The results of these studies indicated that the resonance peaks saturated at different rates which conducted us to propose that at least two different radical species could be used to explain the experimental spectra obtained for DFNa in the present work.

Radiation sensitivities of DFNa, DMPC, DCP, SUR I and CHOL were also studied through the variations of the spectrum area with absorbed radiation dose. It is found to follow a linear function of the type given below where D stands for applied dose in kGy for DFNa. Dose-response curve relevant to I_{pp} signal intensity was calculated to follow a linear function of the type given below where D stands for applied dose in kGy, for SUR I dose-response curve obtained for the intensity measurable without ambiguity and with accuracy, that is, for I_{pp} [= $I(5)$ + $I(11)$] peak to peak intensity is given as an example for these variations below. This curve was found to follow two different linear functions depending on the level of applied dose.

DFNa: $(DI/N)_{normalized} = (0.0046 \pm 0.0015) + (5.38 \pm 0.54)\cdot(D)$, $(R^2 = 0.9898)$

DI = double integral; N = normalization

DMPC: $I_{pp} = (2.226 \pm 0.521) + (0.058 \pm 0.019)\cdot(D)$, $(R^2 = 0.8723)$

DCP: $I_{pp} = (2.423 \pm 0.013) + (0.022 \pm 0.001)\cdot(D)$, $(R^2 = 0.9716)$

SUR I: $I_{low\ doses} = (0.55 \pm 0.08) + (0.013 \pm 0.007)\cdot(D)$, $(R^2 = 0.9012)$

$I_{high\ doses} = (0.13 \pm 0.57) + (0.055 \pm 0.016)\cdot(D)$, $(R^2 = 0.9624)$

CHOL: $I_{pp} = (0.924 \pm 0.123) + (0.074 \pm 0.003)\cdot(D)$, $(R^2 = 0.9856)$

I_{pp} = signal intensity from peak to peak;
$I_{low\ doses}$ = signal intensity from 0–20 kGy;
$I_{high\ doses}$ = signal intensity from 20–50 kGy

Normal condition stabilities of the radical or radicals in an irradiated drug or drug raw material are as important as the radiosensitivity of the materials [25]. Therefore, this feature of the radicals produced in irradiated DFNa samples was also studied. The decay in time of the peak heights of the samples irradiated at different doses and stored at normal and accelerated stability conditions (75% relative humidity, 40 °C) were found to be independent from the irradiation doses. Thus, the peak height decay data obtained for the sample irradiated at a dose of 50 kGy were used to get the decay characteristics of the contributing radicals under both storing conditions. The data relative to the variations of peak height of different resonance peaks showed that in the

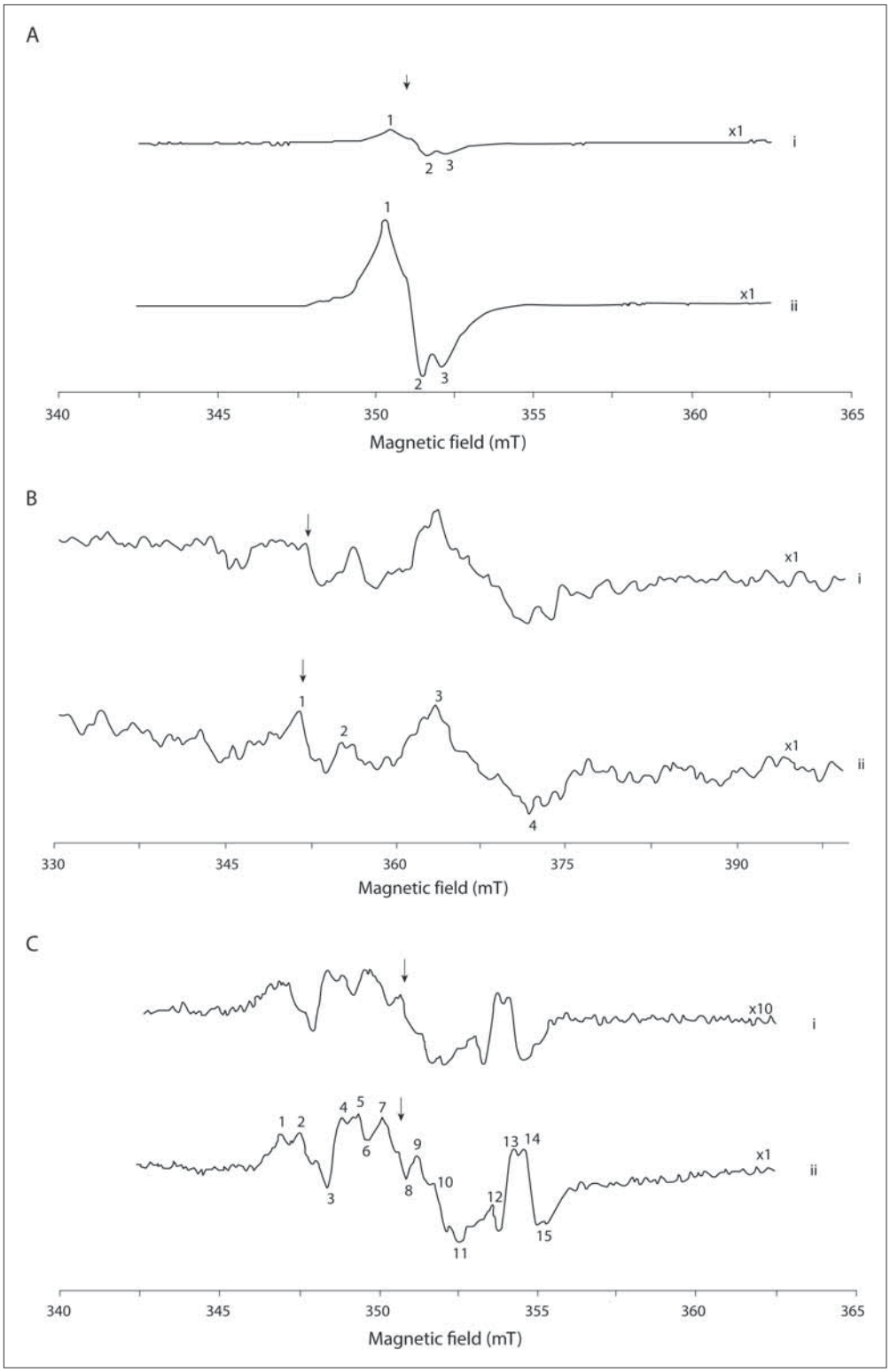

Fig. 1. ESR spectra taken at room temperature of A) DFNa, B) DMPC, C) DCP irradiated at two different dose levels. i) 5 kGy, ii) 50 kGy. Arrows indicate the position of DPPH** line (DPPH: 2,2-diphenyl-1-picrylhydrazyl)

first week the decay was more rapid and over a period of 60 days, 50% of the peak height of a DFNa sample irradiated at dose of 50 kGy, was decayed. Although, all resonance peaks decayed similarly, the 3rd resonance peak was more unstable at normal conditions compared to the other peaks.

The decay with storage time of the peak heights of the DMPC and SUR I samples irradiated at different

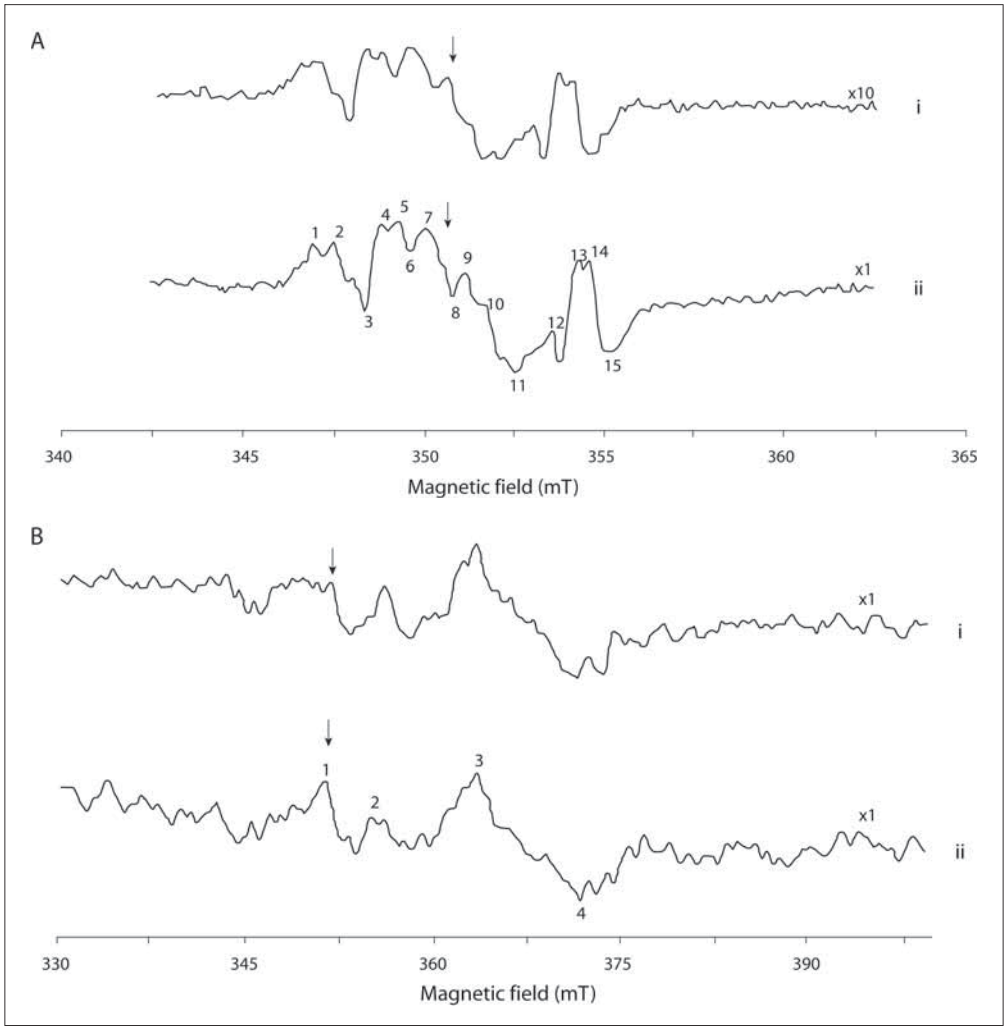

Fig. 2. ESR spectra taken at room temperature of A) SUR I and B) CHOL irradiated at two different dose levels. i) 5 kGy, ii) 50 kGy. Arrow indicates the position of DPPH** line (DPPH: 2,2-diphenyl-1-picrylhydrazyl)

doses and stored at normal and stability conditions were found to be independent from irradiation doses. Thus, the peak height decay data obtained for a sample irradiated at the dose of 50 kGy were used to get the decay characteristics of the contributing radicals under both storing conditions. The data associated with the variations of I_{pp} intensity showed that the species responsible from experimental ESR signal were very unstable at normal conditions. Decay rate of the signal intensity was very rapid during the first hour just after irradiation and 72% decay in the signal intensity was calculated occurring over a storage period of 90 days.

The results of SUR I relevant to I_{pp} [= $I(5)$ + $I(11)$] peak to peak intensity are given below. Two exponential functions decaying with different rates k_1 = 1.39 day^{-1}; k_2 = 0.08 day^{-1} and contributing with different weights were calculated to describe this variation best (I_{01} = 96.9; I_{02} = 61.7). The data associated with the variations of I_{pp} [= $I(5)$ + $I(11)$] peak to peak intensity showed that the decay of the signal intensity was fast and that on the third day of its storage at normal conditions, the signal intensity experienced a decrease of about 57%. Spectra of irradiated samples were observed to begin to transform into the spectrum obtained for unirradiated sample short after irradiation. The decay rates of the peak heights $I(1)$, $I(2)$, $I(3)$, and $I(4)$ were faster when compared with the other resonance peaks. This conclusion was considered as indication of the presence of more than one type of radical species in gamma-irradiated SUR I. In the first week of storage, ESR signal intensities were observed to undergo a decay of about 60%. As is expected, the decays of the contributing radical species were much faster under accelerated stability conditions. Samples of DFNa irradiated at the highest dose (50 kGy) and stored at stability conditions for one day exhibited no ESR signal. The variations of the peak heights of samples irradiated at the dose of 25 kGy and stored at accelerated stability conditions were studied in storage time interval of 15 min–16 h. No ESR signal was detected for the samples stored 12 hours in the accelerated stability con-

Table V Calculated spectral parameters for contributing radical species

Radical species	Relative weight (g)	Line width (G)	g factor
A	0.37	5.36	2.0036
B	0.04	1.91	1.9978
C	0.59	9.56	2.0026

Table VI Results of sterility testing of DFNa, DMPC, DCP, SUR I and CHOL

Substance	Microbiological growth	
	FTM (37 °C)	TDB (25 °C)
DFNa	(–)	(–)
DMPC	(–)	(–)
DCP	(–)	(–)
SUR I	(–)	(–)
CHOL	(–)	(–)

(–): No growth

ditions. The peak height data derived from room temperature ESR spectrum of DFNa sample irradiated at the dose of 50 kGy was used as input for simulation calculations basing on a model of the presence of three radical species with isotropic g factors. The results of these calculations are summarized in *Table V*. The spectra of the contributing radical species (A, B and C) are also given in the same table for comparison.

As can be seen easily the agreement between experimental and theoretical spectra is fairly good which indicates that the model based on three radical species of different characteristic features explaining the experimental ESR spectrum of irradiated DFNa. The line width of radical B is small when compared with the other species. Radical A contributes to the line centered at $g = 2.0036$. A and B radicals are more stable at room temperature. Sterility test results are given in *Table IV* for all formulations.

The decays of the contributing radical species were much faster under accelerated stability conditions compared to the decay at normal conditions. Heights of the studied resonance peaks were absorbed to decay almost to the level of the heights of resonance peaks of an unirradiated sample at the end of first day of storage under accelerated stability conditions. That is, a great part of the species produced after irradiation of DMPC to gamma radiation was decayed over a short storage period. The variations of the peak heights of the samples irradiated at the dose of 50 kGy and stored at accelerated stability conditions were also studied over short storage period that is over a time interval of 15 min–12 h. Similar results were obtained as those for long-term stability studies.

As is expected, the decays of the contributing radical species were much faster under accelerated stability conditions. SUR I samples even those irradiated at the highest dose (50 kGy) kept under accelerated stability conditions for 24 hours were observed to present ESR spectra with peak heights very similar to those measured for unirradiated samples. This result was interpreted as a sign of unstable natures of the radical species produced in gamma-irradiated SUR I under accelerated stability conditions. The variations of I_{pp} [= $I(5)$ + $I(11)$] intensity with storage time under accelerated stability conditions were also studied over the storage period of 15 min to 12 h using samples irradiated at the dose of 50 kGy. The sum of two exponential functions decreasing with different decay constants ($k_1' = 6.96$ min^{-1}, $k_2' = 6.97$ min^{-1}) and with different weights ($I_{01}' = 0.43$; $I_{02}' = 0.42$) were calculated to describe signal intensity decay data obtained for samples stored under accelerated stability conditions.

According to the sterility test results, the negative control showed no growth denoting sterility of the culture medium. The positive control showed bacterial growth signifying suitability of the medium for growth of aerobic and anaerobic bacteria. SAL of DFNa, DMPC, DCP, SUR I and CHOL are 12.4, 17.6, 14.4, 13.8 and 11.9 kGy, respectively. According to the sterility test results there is no microbial growth observed within 2 weeks with both of the media *(Table VI)*. Pyrogen test results which indicate the bacterial endotoxins showed that there was no clothing for all raw materials. The biological results were in agreement with the literature [26–27].

Conclusions

For different radiation dose rates applied as 5, 10 and 25 kGy were chosen as the pharmacopeial-required dose. In order to observe any radiolytic changes in the

samples, 50 kGy was employed as the accelerated condition [7–9, 28].

Physicochemical properties of the irradiated (5, 10, 25, 50 kGy) and unirradiated DFNa, DMPC, DCP, CHOL and SUR I; were examined. Physicochemical properties are organoleptic properties, pH, λ_{max} and melting point. During the experiments, the color of the samples did not change after irradiation. pH values, melting point, spectral properties (except ESR) were determined with some changes which are independent from the radiation dose [3].

According to ESR analysis, a model based on three radical species was found to explain well the experimental results obtained for gamma-irradiated DFNa. Radiation yield of solid DFNa was not high even at the dose of 50 kGy and the radiolytical intermediates produced in DFNa decayed fast at room temperature. The irradiated sample stored in accelerated stability conditions for 12 hours exhibited no ESR signal. This means that solid DFNa and drugs containing DFNa as active ingredient could be safely sterilized by irradiation as the radicals decay only in a day time when it is stored at stability conditions and it has quenched rather fast even if it is stored at normal stability conditions [29, 30].

Although very weak, gamma-irradiated DMPC exhibits ESR signal. This signal is embedded in noise and natural ESR signal of unirradiated DMPC. From variations of the heights of the assigned resonance peaks it was concluded that more than one type of radicals could contribute to the formation of experimental ESR spectrum. However, it was not possible to determine the type of these radicals due to complex nature of recorded experimental spectra.

Radiation yield of solid DMPC was found to be very low even at the applied dose as high as 50 kGy and the life times at normal and accelerated stability conditions of the radical/radicals produced upon irradiation were calculated to be very short. Based on these results, it was concluded that solid DMPC and drug delivery systems containing DMPC as active ingredient could be safely sterilized by gamma irradiation and that ESR technique could be used successfully in monitoring the radiosterilization of this compound [3].

From microwave power saturation and stability results, it was concluded that more than two radicals of different types were produced upon gamma irradiation of SUR I.

Spectrum simulation calculations were not carried out due to complex nature of the experimental spectra.

Signal-to-noise ratio was too low even for samples irradiated even at the highest dose (50 kGy) and therefore, it was concluded that SUR I was not a sensitive compound to gamma irradiation. Besides, radicals produced upon irradiation were unstable under normal and accelerated stability conditions. As expected, the decay of radical species under stability conditions was calculated to be much faster under accelerated stability conditions. Signal intensities of the samples irradiated at the dose of 50 kGy were found to decrease to the levels of the intensities recorded for unirradiated samples.

Thus, it was concluded that solid SUR I could not be sterilized by itself gamma irradiation due to its poor dosimetric feature, but drug delivery systems containing SUR I as active ingredient could be safely sterilized by radiation [3].

All the raw materials, including DFNa, DMPC, DCP and SUR I, can be sterilized safely by gamma irradiation at radiation doses of 12.4 kGy, 17.6 kGy, 14.4 kGy, 13.8 kGy and 11.9 kGy, respectively (below pharmaceutical requirement of 25 kGy) without causing any notable changes. CHOL is the most sensitive substance for the irradiation process in comparison with the others. It might be sterilized below 10 kGy and further studies should be carried out to evaluate the effect of irradiation on the function of CHOL. The radicals of raw materials produced upon irradiation were unstable under normal and accelerated stability conditions indicating that gamma radiation sterilization can be used as an effective method for the sterilization of those substances.

As conclusion, this study showed that DFNa, DMPC, DCP, SUR I and CHOL can be sterilized with lower doses, namely 12.4 kGy, 17.6 kGy, 14.4 kGy, 13.8 kGy and 11.9 kGy, respectively. The confirmation and the validation of the SAL doses which are below 25 kGy is a big advantage to the researcher or to the customer in order to save money and time, because sterilization is achieved by the irradiation of the products in relation with time. Using lower doses instead of 25 kGy means the reduction of irradiation time. The sterilization fee is paid due to the sterilization time, thus, lower SAL values cost less money. This study showed that gamma radiation sterilization is a reliable sterilization method for a wide range of raw materials.

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References

1. Bushell JA, Claybourn M, Wiliams HE, Murphy DM: An EPR and ENDOR study of γ- and β-radiation sterilization in poly (lactide-co-glycolide) polymers and microspheres. J Control Rel 110, 49–57 (2005)
2. Mohammed AR, Bramwell VW, Coombes AGA, Perrie Y: Lyophilisation and sterilization of liposomal vaccines to produce stable and sterile products. Methods 40, 30–38 (2006)

3. Erdoğan S, Özer AY, Ekizoğlu M, Özalp M, Çolak Ş: Gamma irradiation of liposomal phospholipids. FABAD J Pharm Sci 31, 182–190 (2006)
4. Olguner Mercanoğlu G, Özer AY, Çolak Ş, Korkmaz M, Özalp M, Ekizoğlu M, Barbarin N, Tilquin B: Radiosterilization of sulfonamides I: Determination of the effects of gamma irradiation on solid sulfonamids. Rad Phys Chem 69, 511–520 (2004)
5. Botelho ML, Verde SC, Alves L, Balchior A, Reymao J, Trabulo S, Gaspar MM, Cruz MEM, Simoes S: Radiation sterilization of antibiotic liposome formulations: A case study. Rad Phys Chem 76, 1542–1546 (2007)
6. Berk F, Özer AY: Radiation sterilization of medical devices. FABAD J Pharm Sci 24, 223–231 (1999)
7. Berk F: Tek Kullanımlık tıbbi malzemelerin gama radyasyonu ile sterilizasyonu ve diğer yöntemlerle karşılaştırılması. MSci thesis, Institute of Health Science, Hacettepe Universty, Ankara, Turkey (2002)
8. Liman V, Özer AY, Çolak S, Korkmaz M, Kılıç E, Özalp M: The effects of gamma radiation sterilization on cefazolin sodium. FABAD J Pharm Sci 30, 124–132 (2005)
9. Naki N: Kozmetikler ve kozmetik hammaddelerinin gama radyasyonla sterilizasyonu/dekontaminasyonu üzerinde çalışmalar. MSci thesis, Institute of Health Science, Hacettepe University, Ankara, Turkey (2003)
10. Olguner G: Radiation sterilization of sulfonamides and comparison with the other sterilization techniques. MSci thesis, Institute of Health Science, Hacettepe University, Ankara, Turkey (2000)
11. Türker S, Erdoğan S, Özer AY, Ergün EL, Tuncel M, Bilgili H, Deveci S: Scintigraphic imaging of radiolabelled drug delivery systems in rabbits with arthritis. Int J Pharm 296, 34–43 (2005)
12. Zuidam NJ, Gouw HK, Barenholz Y, Crommelin DJ: Physical (in) stability of liposomes upon chemical hydrolysis: The role of lysophosphalipids and fatty acids. Biochim Biophys Acta 22, 101–110 (1995)
13. Barbarin N, Tilquin B: Study of nonvolatile degradation compounds produced by radiosterilization of cefatoxime. Radiat Phys Chem 60, 359–367 (2001)
14. Gibella M, Crucq AS, Tilquin B, Stocker P, Lesgards G, Raffi Y: ESR studies of some irradiated pharmaceuticals. Radiat Phys Chem 58, 69–76 (2000)
15. Olguner Mercanoğlu G, Özer AY, Özalp M, Ekizoğlu M: Radiosterilization of sulfonamides II: Determination of the effects of gamma irradiation on commercial sulfanomide preparations. Turkish J Pharm Sci 4, 159–170 (2007)
16. British Pharmacopoeia (2004): University Press, Cambridge
17. Kantaglu O (2004): Endüstride sterilizasyon kursu, TAEK, Ankara
18. Phillips GO, Power DM, Sewart MCG: Effects of gamma irradiation on sodium sulphacetamide. Radiat Res 46, 236–250 (1971)
19. Amin GAM, Spyrou NM: Study of gamma-radiation-induced optical effects in Ge-Se-Cd for possible industrial dosimetric applications. Rad Phys Chem 72, 419–422 (2007)
20. Pandey BN, Lathika KM, Mishra KP: Radiation induced oxidative damage modification by cholesterol in liposomal membrane. Rad Phys Chem 54, 481–489 (1999)
21. Pandey BN, Lathika KM, Mishra KP: Modification of radiation-induced oxidative damage in liposomal and microsomal membrane by eugenol. Rad Phys Chem 75, 384–391 (2006)
22. Plikk P, Odelius K, Hakkarainen M, Albertsson AC: Finalizing the properties of porous scaffolds of aliphatic polyesters through radiation sterilization. Biomaterials 27, 5335–5347 (2006)
23. Sprinz H, Schwinn J, Naumov S, Brede O: Mechanism of thiyl radical-catalyzed isomerization of unsaturated fatty acid residues in homogeneous solution and in liposomes. BBA 1483, 91–100 (2000)
24. Varshney L, Dodke PB: Radiation effect studies on anticancer drugs, cyclophosphamide and doxorubicin for radiation sterilization. Rad Phys Chem 71, 1103–1111 (2004)
25. Deeley CM: A basic interpretation of the technical language of radiation processing. Rad Phys Chem 71, 503–507 (2004)
26. El-Ridy MS, Mostafa DM, Shehab A, Nasr EA, El-Alim SA: Biological evaluation of pyrazinamide liposomes for treatment of Mycobacterium tuberculosis. Int J Pharm 330, 82–88 (2007)
27. Katusin-Razem B, Mihaljevic B, Razem D: Microbial decontamination of cosmetic raw materials and personal care products by irradiation. Rad Phys Chem 66, 309–316 (2003)
28. Dam AM, Gazso LG, Kaewpila S, Maschek I: Radiation treatment of pharmaceuticals. Radiat Chem 47, 515–517 (1996)
29. Da Costa ZM, Pontusehka WM, Campos LL: Study of the ESR signal of gamma irradiated hydroxyapatite for dose assessment. Nucl Instrum Meth B 218, 283–288 (2004)
30. Faucitano A, Buttafava A, Montanari L, Cilurzo F, Conti B, Genta I, Valvo L: Radiation-induced free radical reactions in polymer/drug systems for controlled release: An EPR investigation. Radiat Phys Chem 67, 61–72 (2003)