

Gamma-irradiated liposome/niosome and lipogelosome/niogelosome formulations for the treatment of rheumatoid arthritis

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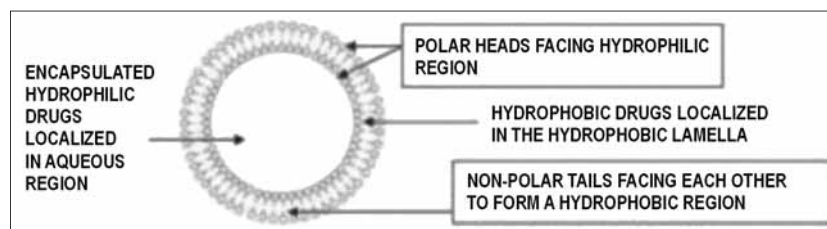
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Abstract: Treatment of rheumatoid arthritis by intraarticular administration of anti-inflammatory drugs encapsulated in drug delivery systems, such as liposomes/niosomes and lipogelosomes/niogelosomes, prolongs the residence time of the drugs in the joint. It was therefore anticipated that liposome/niosome entrapment would enhance the efficacy of drugs in the inflammatory sides. Liposomes are good candidates for the local delivery of therapeutic agents, such as diclofenac sodium (DFNa), for intraarticular delivery. Drugs for parenteral delivery must be sterile, and radiation sterilization is a method recognized by pharmacopoeias to achieve sterility of drugs. However, irradiation might also affect the performance of drug delivery systems. One of the most critical points is irradiation dose, because certain undesirable chemical and physical changes may accompany with the treatment, especially with the traditionally applied dose of 25 kGy. The present study aims to determine the effects of gamma irradiation on DFNa-loaded liposomes/niosomes and lipogelosomes/niogelosomes for the treatment of rheumatoid arthritis.



Niosome structure

Keywords: diclofenac sodium, gamma irradiation, sterilization, liposomes, niosomes, lipogelosomes, niogelosomes, rheumatoid arthritis

Introduction

Liposomes are phospholipid bilayer membrane-bound vesicles that can encapsulate a wide variety of substances either within their lipid membranes or their central aqueous cores [1]. Gamma irradiation is an approved sterilization technique for some pharmaceuticals [2–10] and has turned out to be an interesting and promising technique also for the sterilization of liposomes. Zuidam et al. [11] have published a review of the different sterilization techniques for liposomes, and they concluded that gamma irradiation can be accepted as a safe and convenient sterilization technique for liposomes, and more

studies are necessary. The use of gamma rays for the sterilization of pharmaceutical raw materials and dosage forms is an alternative method for sterilization [12]. Currently, gamma irradiation includes high penetrating power, low chemical reactivity, low measurable residues, and small temperature rise. A minimum absorbed dose of 25 kGy is regarded as adequate for the purpose of sterilizing pharmaceutical products without providing any biological validation [13]. However, chemical degradation of the phospholipids may take place during gamma irradiation, and production of new radiolytic products during the irradiation process is one of the major problems of the radiosterilization. Therefore, the principal problem in radiosterilization is to determine

and to characterize these physical and chemical changes originating from high-energy radiation [12]. Stark [14] and Albertini and Rustichelli [15] presented reviews summarizing the effects of gamma irradiation on lipids and liposomal structure. In some studies, peroxidation of unsaturated phospholipids and formation of lysophospholipids, free fatty acids, phosphatidic acid, and different hydrocarbon compounds have been observed [16]. Efforts have been done to reduce the degradation by addition of radical scavengers or by freezing and lyophilization [17, 18]. The physical properties of gamma-irradiated liposomes (size, bilayer rigidity, and permeability) are less sensitive to changes than the chemical structure [17, 19]. Furthermore, there has been a reported increased physical stability due to a resulting increased electrostatic repulsion between the liposomes preventing especially the neutral liposomes from aggregation and fusion [17, 20]. In this study, the effects of gamma irradiation liposome/niosome and lipogelosome/niogelosome formulations prepared for the purpose of RA treatment are investigated in more detail. Physical/chemical analyses (organoleptic controls, pH, particle size, viscosity, *in vitro* release, and electron spin resonance [ESR] analysis) of the formulations are followed by microbiological characterization (sterility, apyrogenicity, and sterility assurance level [SAL] determination) of the subsequent produced formulations. Furthermore, stability tests were carried out under normal (60% relative humidity and 25 °C temperature) and accelerated (75% relative humidity and 40 °C temperature) test conditions for 3 months.

This work complies information about the studies developed in order to find out if gamma radiation could be applied as a sterilization method of liposome/niosome and lipogelosome/niogelosome formulations containing diclofenac sodium (DFNa).

Materials and Methods

Dimyristoyl phosphatidylcholine (DMPC), diclofenac sodium (DFNa), and SUR I (hexa decyl 3-polyglycerole) (L'Oreal) were kindly provided by Nattermann Phospholipids (Köln, Germany), Deva (Istanbul, Turkey), L'oreal (France), respectively. Dicapryl phosphate (DCP) and cholesterol (CHOL) were obtained from Sigma Chemical Co. (St. Louis, USA). Chloroform was purchased from Merck (Germany). All other chemicals were of analytical grade.

All investigations including (organoleptic properties, TLC, pH, viscosity, *in vitro* release, ESR studies, and microbiological tests) were performed on formulations 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.

Irradiation procedure

All irradiations were performed under normal conditions (25 °C, 60% relative humidity) in dark using a ⁶⁰Co 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.

Preparation of liposomes/niosomes

Liposome and niosome dispersions were prepared by film technique [21]. Briefly, liposomes and niosomes were prepared by dissolving 40 μmol·mL⁻¹ of phospholipids and/or the surfactant in 30-mL chloroform in a round-bottom flask. The chloroform was removed using a rotary evaporator (Buchi 461, Switzerland) under reduced pressure to form a thin film (7:1:2 molar ratio) over the wall of the flask. The dried film was then hydrated over a water bath with 10 mM HEPES (pH 7.4) containing 10 mg·mL⁻¹ DFNa [8].

Preparation of lipogelosomes/niogelosomes

Lipogelosome and niogelosome formulations were prepared by incorporation of liposomes and niosomes in structured vehicles. Carbopol 940 (C 940) at the concentration of 1% (*w/v*) in distilled water [22] and CMC-Na (carboxymethylcellulose sodium) at the concentration of 2.5% (*w/v*) [23] were used as gel-forming agents because of their good bioadhesive properties. For the preparation of lipogelosomes/niogelosomes, gel formulations of C 940 and CMC-Na and liposomal/niosomal DFNa were mixed in 1:1 ratio on weight basis (Table I) [8].

Table I Summary of the liposome/niosome and lipogelosome/niogelosome formulations

Formulation	Composition	Ratio	Code
Liposome	(DMPC-CHOL-DCP)	(7:1:2)	L
Niosome	(SUR I-CHOL-DCP)	(7:1:2)	N
Lipogelosome	(DMPC-CHOL-DCP) + C-940	(7:1:2) + (1:1) (<i>w/w</i>)	LJ
Niogelosome	(SUR I-CHOL-DCP) + C-940	(7:1:2) + (1:1) (<i>w/w</i>)	NJ

The summary of DFNa-loaded lipogelosome and niogelosome formulations are given in *Table I*.

Organoleptic properties

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

TLC studies

Samples of 20 $\mu\text{g}\cdot\text{mL}^{-1}$ liposome, niosome, lipogelosome, or niogelosome dispersions were dropped to the GF 254 silikagel plates which were activated at 110 °C for 60 min in an oven. The plates were dried and developed in chloroform–methanol–water (65:25:4) solvent system. Formulations were made visible by immersing the plates into iodine tank, the spots were determined under UV lamp at 254 nm before and after gamma irradiation.

pH measurements

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

Particle size measurement

Mean particle size and size distributions of the liposome and niosome dispersions were measured by dynamic light scattering method (Malvern Mastersizer 2000, UK) before and after irradiation.

Determination of encapsulation efficiency

The preparation of liposome/niosome formulations containing DFNa was described in Section 2.2. After the hydration of dried film with 10-mM HEPES (pH 7.4) containing 10 $\text{mg}\cdot\text{mL}^{-1}$ DFNa, free DFNa was removed by centrifugation three times at 17,500 rpm for 45 min in each. The pellets that were obtained after the centrifugations were treated with detergent (Triton X-100 in 10 mM HEPES [pH 7.4] buffer), and then final clear solutions were analyzed for drug content spectrophotometrically at $\lambda = 303$ nm. Encapsulation efficiency was calculated as a fraction of drug in the liposome/niosome pellets expressed as the percentage of the total drug content. The *in vitro* characterization and *in vitro* release studies are given in our previous studies [8].

Determination of liposomal phospholipid

Determination of lipid content gives an idea about the efficiency of the method of preparation of liposomes. Liposomal phospholipid content was determined by the colorimetric method of Rouser et al. [24].

In vitro release of DFNa from liposome and niosome formulations

In vitro release of DFNa from liposome and niosome formulations was measured spectrophotometrically (Schimadzu UV 160A, Japan) at $\lambda = 275$ nm by incubation of 0.1 mL of liposome and niosome formulations in 10 mL 10 mM HEPES (pH 7.4) buffer at 37 °C in mild shaking water bath, before and after irradiation. Samples were withdrawn at fixed time intervals, and no interference of the empty formulations was detected at this wavelength.

In vitro release of DFNa from lipogelosome and niogelosome formulations

In vitro release properties of liposome and niosome dispersions were evaluated by using Franz-type diffusion cell before and after irradiation. About 0.5 g of sample was introduced into a donor compartment separated by a cellophane membrane (Thomas Sci. Comp., USA) from the receptor compartment, 20 mL of HEPES buffer (pH 7.4). The whole assembly was placed in a water bath, maintained at 37 °C and continuously well stirred. Care was paid to remove any air bubble from the under side of the membrane and the receiving solution. One-milliliter samples were removed from the receiver compartment at specified time intervals, i.e., partial sampling and refilled with an equal volume fresh buffer. All samples were analyzed for DFNa content spectrophotometrically at the $\lambda = 275$ nm.

Viscosity measurements

Viscosity measurements of lipogelosomes and niogelosomes were performed by using rheometer (Brookfield, USA) at 25 °C and 37 °C. The shear rate increased from 0 to 100 rpm. The viscosity was determined from the flow curve obtained at different values of shear rate.

ESR measurements

ESR measurements of DFNa-loaded formulations were carried out using Bruker EMX 113 spectrometer operating at 9.5 Ghz. The spectrophotometer operating

Table II 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	6.3×103
Sweep time	83.89 s
Time constant	327.68 s
Conversion time	81.92 s
Temperature	RT (room temperature)

conditions adapted during the experiments are given in *Table II*.

Sterility test

For the sterility test, two media were used [fluid thioglycolate medium (FTM) and soybean-casein digest medium (SCDM)]. One hundred microliters of the formulations was inoculated to FTM and SCDM medium. They were incubated for 14 days at 35 °C, respectively. After 14 days, the tubes that are turbid were considered as non-sterile, and the tubes that are clear were considered as sterile.

Pyrogen (LAL) test

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

SAL determinations

The formulations 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 (tryptic soy broth) plates at 35–37 °C for 2 weeks. About 1 kGy ir-

radiation dose was added to SAL determination experiments for obtaining the microorganism death graphics. *Bacillus pumilus* colonies were enumerated, and cfu in 1 mL were calculated. SAL 10⁻⁶ dose was calculated from the logarithmic microorganism death graphics.

Stability tests

For stability testing, irradiated samples (5, 10, 25, and 50 kGy) were stored at accelerated (60% relative humidity, 25 °C temperature) and normal (4–8 °C) conditions protected from light and in well-sealed containers for 3 months. Particle size, encapsulation efficiency, and phospholipid content were monitored under stability test conditions.

Results and Discussion

Liposomes are currently under investigation in various fields of science and technology, being used as a model system of membrane structure and function and in numerous applications in agriculture, food industry, drug delivery systems, and DNA transfection [19]. The DFNa-loaded formulations coated L, N, LJ, and NJ are formulated for the purpose of intraarticular delivery, which requires sterile and apyrogenic injection. Irradiation is an established method of sterilization for pharmaceutical products. Gamma radiation, characterized by deep penetration and low dose rates, effectively kills microorganisms throughout the pharmaceutical formulations, products, and their packaging. However, sterilization of these systems has been problematic, with degradation of the liposomes being reported after sterilization using the various techniques available [25, 26]. This study describes the investigation of the effects of ionizing radiation to liposome/niosome and lipogelosome/nioigelosome formulations containing DFNa following gamma irradiation.

Color change in the irradiated substances is the simplest and most helpful observation to get information about possible radiolytic intermediates produced in these substances upon irradiation. As no color change was observed in the irradiated formulations in the applied dose region of 5–50 kGy, it can be concluded that

Table III TLC results of formulations before and after gamma irradiation

Formulation	R_F				
	Dose rate (kGy)				
	0	5	10	25	50
L	0.327 ± 0.045	0.333 ± 0.051	0.334 ± 0.048	0.340 ± 0.053	0.343 ± 0.045
N	0.412 ± 0.036	0.424 ± 0.055	0.427 ± 0.043	0.433 ± 0.035	0.437 ± 0.039
LJ	0.311 ± 0.019	0.323 ± 0.021	0.329 ± 0.023	0.331 ± 0.031	0.335 ± 0.041
NJ	0.407 ± 0.032	0.411 ± 0.019	0.417 ± 0.013	0.421 ± 0.023	0.427 ± 0.024

Table IV Measured pH values for control and irradiated formulations (*n*: 6)

Formulation	pH				
	Dose rate (kGy)				
	0	5	10	25	50
L	7.45 ± 0.01	7.40 ± 0.02	7.38 ± 0.02	7.24 ± 0.02	7.22 ± 0.01
N	7.40 ± 0.03	7.40 ± 0.03	7.39 ± 0.01	7.23 ± 0.01	7.22 ± 0.02
LJ	7.42 ± 0.01	7.40 ± 0.02	7.40 ± 0.03	7.30 ± 0.03	7.23 ± 0.02
NJ	7.39 ± 0.04	7.41 ± 0.02	7.37 ± 0.02	7.31 ± 0.02	7.29 ± 0.01

either radiolytic intermediates were not produced by irradiation in studied samples or formed intermediates do not exhibit any absorption in the visible region. The negative result in color change of the present work was consistent with the results reported in the literature for similar compounds [12]. With regard to the physical characteristics, there were no significant changes in the organoleptic properties of the formulations before or after irradiation. These results are in agreement with the previous studies [25].

R_F values determined by TLC method of control and irradiated samples were found to be significantly different ($p < 0.05$) and are given in *Table III*. R_F values of formulation changed with the increasing radiation dose levels, which might be due to the presence of degradation products originating from phospholipids and other components in the formulations. The degradation products cannot be identified with TLC analysis, further an-

alytical methods, such as HPLC, might be studied to identify the degradation products.

Experimental results showed that pH of all the formulations decreased significantly ($p < 0.05$) (*Table IV*). The lower pH measured after irradiation of the formulations might be caused by the degradation of the phospholipids and the formation of acidic degradation products, such as distearoylphosphatidic acid (DSPA) and fatty acids [17].

The size of the liposome and niosome formulations was not affected by gamma irradiation as determined by dynamic light scattering. The particle size of irradiated and non-irradiated formulations was essentially the same (*Table V*).

The encapsulation efficiency of L and N formulations did not change as shown in *Table VI*; however, phospholipid content of L formulation was decreased with gamma irradiation (*Table VII*). That change in phospholipid

Table V Particle size and distribution results for control and irradiated formulations (*n*: 6)

Formulation	Particle size (nm) ^a				
	Dose rate (kGy)				
	0	5	10	25	50
L	239 ± 0.011	235 ± 0.010	237 ± 0.014	241 ± 0.017	240 ± 0.015
N	279 ± 0.012	276 ± 0.012	281 ± 0.010	273 ± 0.009	277 ± 0.008

^aL and N formulations were dispersed in gel formulations that is why the LJ and NJ results were the same with the L and N formulations

Table VI Encapsulation efficiency results for control and irradiated formulations (*n*: 6)

Formulation	Encapsulation efficiency (%) ^a				
	Dose rate (kGy)				
	0	5	10	25	50
L	10.8 ± 0.1	10.9 ± 0.1	10.5 ± 0.5	10.9 ± 0.4	10.8 ± 0.2
N	9.6 ± 0.1	9.7 ± 0.2	9.6 ± 0.1	9.9 ± 0.2	9.3 ± 0.1

^aL and N formulations were dispersed in gel formulations that is why the LJ and NJ results were the same with the L and N formulations

Table VII Phospholipid contents of control and irradiated formulations (*n*: 6)

Formulation	Phospholipid content (%) ^a				
	Dose rate (kGy)				
	0	5	10	25	50
L	97.3 ± 2.2	95.3 ± 3.1	94.5 ± 0.5	92.9 ± 2.4	88.8 ± 1.3

^aL formulation was dispersed in gel that is why the LJ results were the same with the L formulations

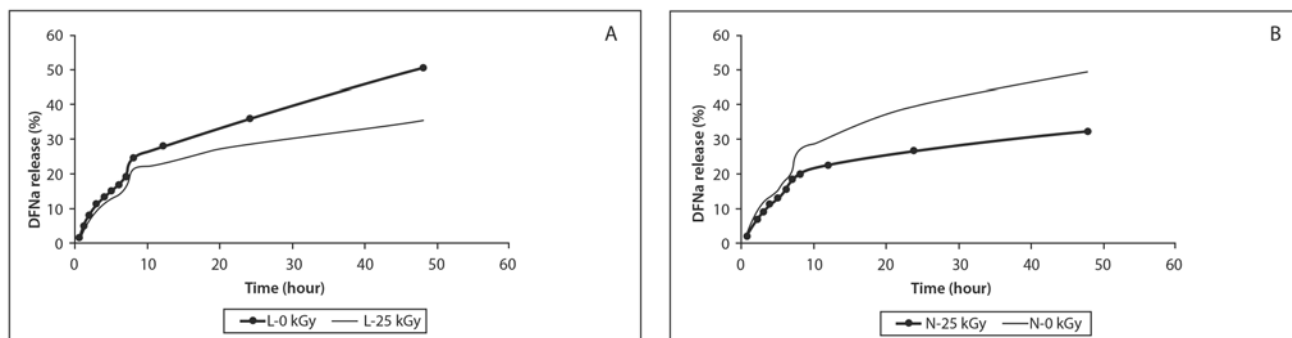


Fig. 1. In vitro release of DFNa from L (A) and N (B) formulations

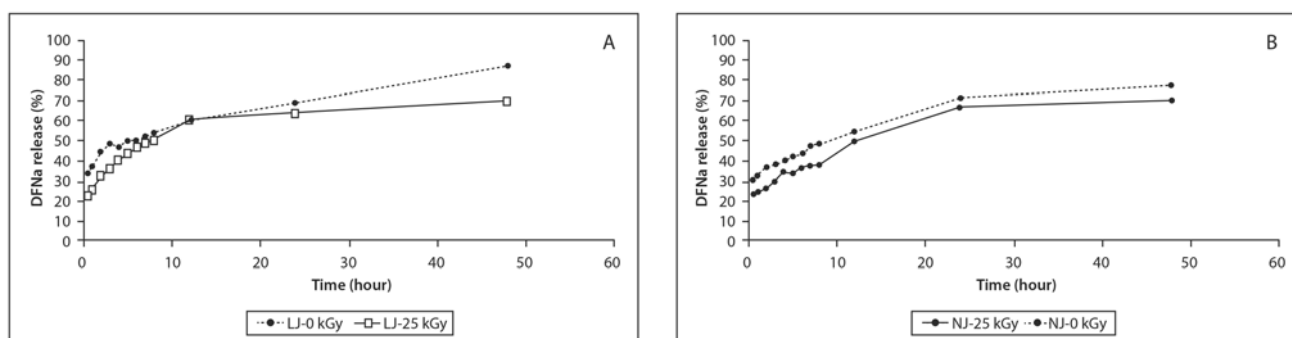


Fig. 2. In vitro release of DFNa from LJ (A) and NJ (B) formulations

content might be a sign of the onion peeling effect. All results were in agreement with each other.

The drug release profiles of DFNa from the formulations were examined in 10 mM HEPES (pH = 7.4) buffer. The total amount of DFNa was released in 48 h from the formulations. As is expected, liposome and niosome formulations showed higher release of DFNa than lipogelosome and niogelosome formulations. The lipogelosome and niogelosome formulations significantly ($p < 0.05$) prolonged the DFNa release compared with the unirradiated and irradiated formulations (Figs 1 and 2). The release of DFNa from the lipogelosome and niogelosome formulations is a combination of the release of free, surface-bound, and encapsulated

drug through the micellar network channel structures of the gel. Also, the release rate of a drug dissolved in the liquid phase of lipogelosome and niogelosome formulations may be affected by the type and concentration of the gelling agent and by the processing conditions [27]. Release profiles of the L, N, LJ, and NJ formulations were altered after irradiation. The release rates were significantly increased with the irradiation process. These results might imply radiation-induced decrease in bilayer fluidity. The changes in permeability and fluidity in the bilayer were found to be dependent on the radiation dose in a biphasic fashion in previous studies, and our results are in agreement with the literature [25].

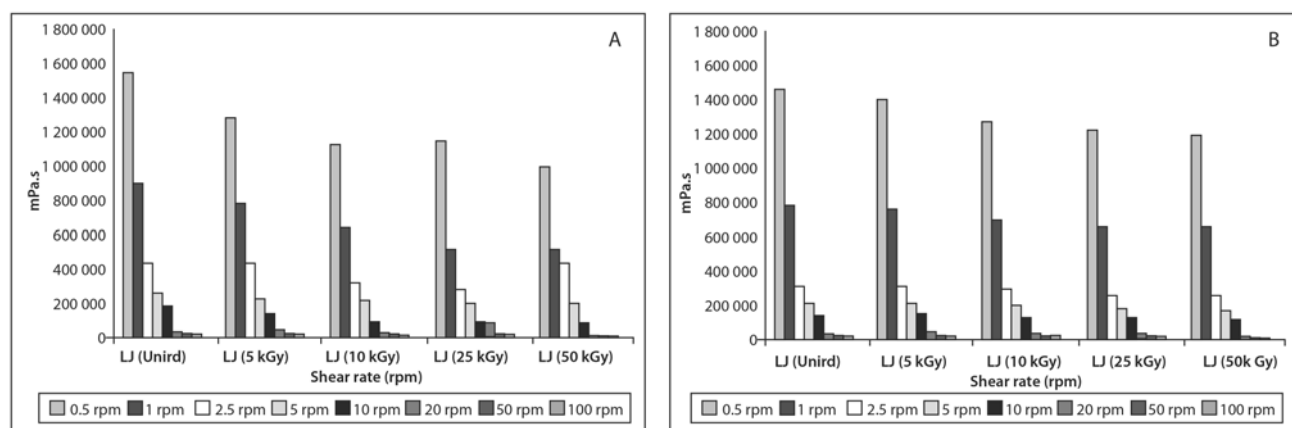


Fig. 3. Rheological behavior (viscosity) of DFNa-loaded LJ formulation before and after gamma irradiation at (A) 25 °C and (B) 37 °C

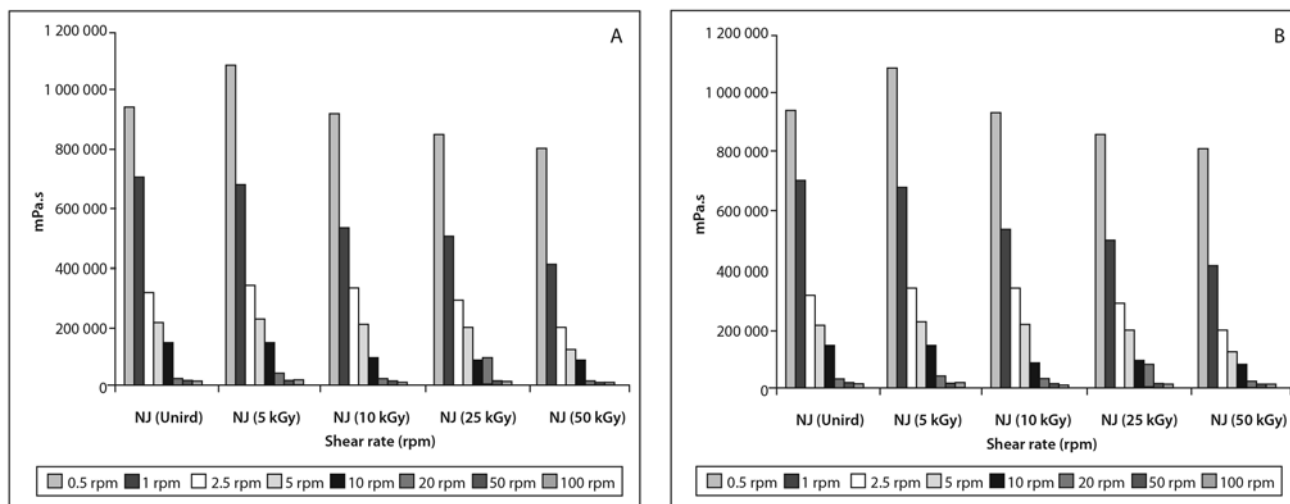


Fig. 4. Rheological behavior (viscosity) of DFNa-loaded NJ formulation before and after gamma irradiation at (A) 25 °C and (B) 37 °C

Lipogelosome and niogelosome formulations showed a higher dynamic viscosity than liposome and niosome formulations due to the presence of the micellar network structure of gel in lipogelosomes and niogelosomes. As the shear rate increased, the viscosity of the lipogelosome and niogelosome formulations decreased. All the formulations displayed a non-Newtonian behavior at both 25 °C and 37 °C [8]. The difference between the viscosities of lipogelosome and niogelosome formulations was found to be statistically significant ($p < 0.05$). The dynamic viscosity of the lipogelosome and niogelosome formulations was significantly lowered upon irradiation process (Figs 3 and 4). These changes might be responsible for the shortening of polymer chains and structural degradation, depending upon the irradiation dose levels.

As is expected, unirradiated and irradiated formulations (liposome, niosome, lipogelosome, and niogelosome) were observed to be not exhibiting ESR signals. It is likely due to the unstable natures of the radical species

Table VIII Results of sterility test of liposomes/niosomes and lipogelosomes/niogelosomes

Formulation	Microbiological growth	
	FTM (37 °C)	SCDM (25 °C)
L	(-)	(-)
N	(-)	(-)
LJ	(-)	(-)
NJ	(-)	(-)

(-): no growth

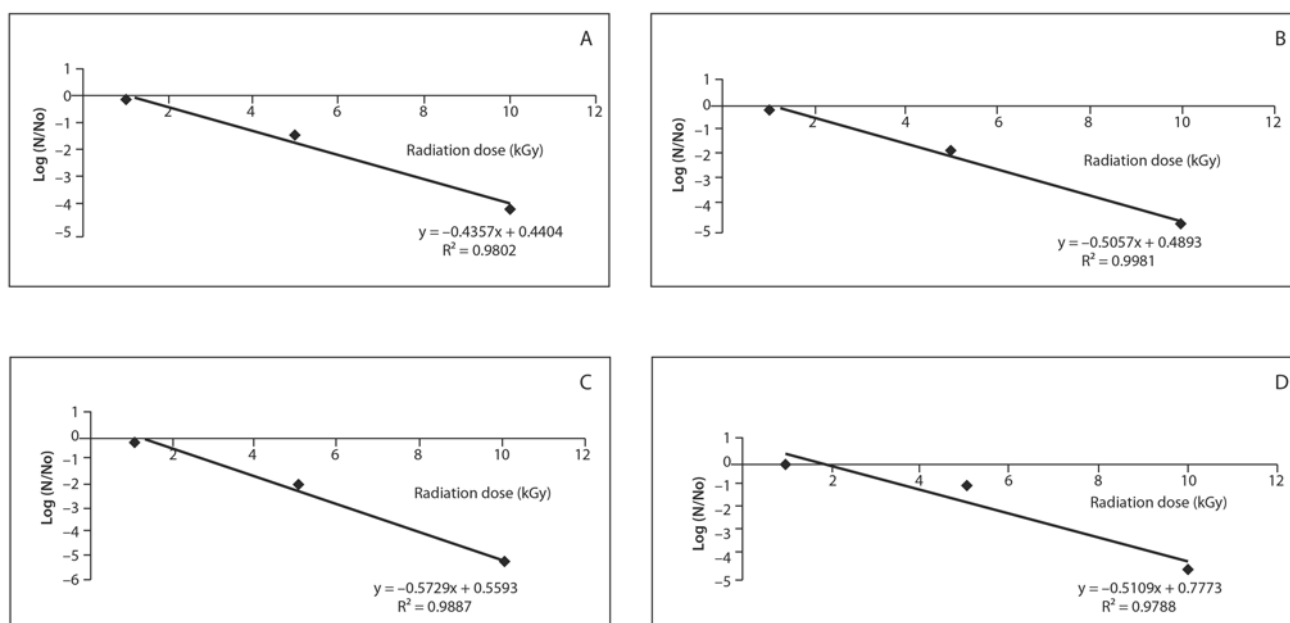


Fig. 5. Microorganism death graphics (SAL). (A) L, (B) N, (C) LJ, (D) NJ

produced upon gamma irradiation in solutions where radical-radical recombination is very fast.

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. According to the sterility test results, there was no microbial growth observed within 2 weeks with both of the media (Table VIII).

Pyrogen test results which indicate that the bacterial endotoxins (most common pyrogens) showed there was no clothing for all raw materials.

SAL of L, N, LJ, and NJ were found as 13.8, 15.2, 14.3, and 14.9 kGy, respectively (Fig. 5).

Those results showed that the coded formulations might be sterilized with the lower radiation doses after the validation of gamma irradiation, in comparison with the recommended radiation dose of 25 kGy in pharmacopeias.

According to the stability studies performed at room (25 °C) and refrigerator (4 °C) temperatures, the particle size distribution of L and N formulations did not change with gamma irradiation and stability test conditions (Table IX). Encapsulation of unirradiated L formulations did not change as the irradiated ones in both of

the stability conditions up to 1 month. However, the encapsulation efficiency of L formulation significantly decreased at room temperature in comparison with the formulations which were kept at 4 °C (Table X). The reason for those results might be the effect of gamma irradiation which might be more intensive at higher temperatures. According to the encapsulation efficiency results of N formulation, gamma irradiation effect on N formulation was higher than L formulation, the encapsulation efficiency results were significantly lower. The phospholipid content of the unirradiated formulations did not change at 4 °C; however, the phospholipid content of the irradiated formulations decreased ($p < 0.05$) over 3 months (Table XI). The stability test might be useful for the determination of optimum sterilization conditions with gamma irradiation for DFNa-loaded formulations.

Conclusions

In this study, the effectiveness of gamma irradiation on DFNa-loaded formulations, L, N, LJ, and NJ, was investigated. Our results indicated that organoleptic proper-

Table IX ■ Stability test results for particle size distribution of unirradiated and irradiated formulations

Time (month)	Particle size (nm) ^a									
	Refrigerator (4 °C)					Room temperature (25 °C)				
	Formulation					Formulation				
	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)
1	235 ± 0.013	233 ± 0.015	235 ± 0.011	231 ± 0.011	230 ± 0.014	238 ± 0.013	230 ± 0.011	229 ± 0.010	234 ± 0.013	235 ± 0.016
2	234 ± 0.010	228 ± 0.010	231 ± 0.010	227 ± 0.010	232 ± 0.016	233 ± 0.010	234 ± 0.012	233 ± 0.014	237 ± 0.012	234 ± 0.014
3	230 ± 0.011	232 ± 0.014	231 ± 0.010	229 ± 0.012	235 ± 0.010	235 ± 0.014	233 ± 0.011	237 ± 0.015	230 ± 0.011	235 ± 0.012
	N	N (5 kGy)	N (10 kGy)	N (25 kGy)	N (50 kGy)	N	N (5 kGy)	N (10 kGy)	N (25 kGy)	N (50 kGy)
1	272 ± 0.015	271 ± 0.011	270 ± 0.010	271 ± 0.013	266 ± 0.010	275 ± 0.010	276 ± 0.011	271 ± 0.010	275 ± 0.010	277 ± 0.011
2	275 ± 0.012	270 ± 0.013	275 ± 0.011	273 ± 0.012	273 ± 0.015	269 ± 0.012	271 ± 0.012	269 ± 0.012	274 ± 0.013	272 ± 0.017
3	271 ± 0.014	275 ± 0.014	277 ± 0.015	279 ± 0.010	279 ± 0.012	271 ± 0.018	273 ± 0.015	272 ± 0.016	271 ± 0.010	275 ± 0.011

^aL and N formulations were dispersed in gel formulations that is why the LJ and NJ results were the same with the L and N formulations

Table X ■ Stability test results for encapsulation efficiency of unirradiated and irradiated formulations

Time (month)	Encapsulation efficiency (%) ^a									
	Refrigerator (4 °C)					Room temperature (25 °C)				
	Formulation					Formulation				
	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)
1	10.8 ± 0.3	10.7 ± 0.1	10.7 ± 0.5	10.5 ± 0.4	10.6 ± 0.2	10.6 ± 0.1	10.5 ± 0.3	10.5 ± 0.1	10.4 ± 0.4	10.6 ± 0.3
2	10.7 ± 0.5	10.0 ± 0.8	10.5 ± 0.3	10.1 ± 0.6	10.2 ± 0.1	10.3 ± 0.2	8.9 ± 0.2	10.1 ± 0.3	9.9 ± 0.1	10.2 ± 0.1
3	10.7 ± 0.4	9.3 ± 0.4	10.0 ± 0.5	9.9 ± 0.2	9.8 ± 0.1	10.4 ± 0.2	8.1 ± 0.1	9.5 ± 0.5	8.6 ± 0.2	8.8 ± 0.4
	N	N (5 kGy)	N (10 kGy)	N (25 kGy)	N (50 kGy)	N	N (5 kGy)	N (10 kGy)	N (25 kGy)	N (50 kGy)
1	9.6 ± 0.1	9.5 ± 0.1	9.6 ± 0.1	9.7 ± 0.2	9.4 ± 0.1	9.6 ± 0.2	9.7 ± 0.2	9.5 ± 0.3	9.3 ± 0.4	9.1 ± 0.1
2	9.5 ± 0.5	9.3 ± 0.1	9.4 ± 0.6	9.2 ± 0.4	9.1 ± 0.5	9.6 ± 0.2	9.3 ± 0.3	9.1 ± 0.1	9.0 ± 0.2	9.0 ± 0.1
3	9.5 ± 0.3	9.1 ± 0.3	9.0 ± 0.1	9.0 ± 0.4	9.0 ± 0.2	9.0 ± 0.2	8.7 ± 0.2	8.7 ± 0.1	8.6 ± 0.2	8.7 ± 0.4

^aL and N formulations were dispersed in gel formulations that is why the LJ and NJ results were the same with the L and N formulations

Table XI Stability test results for phospholipid content of unirradiated and irradiated formulations

Time (month)	Phospholipid content (%) ^a									
	Refrigerator (4 °C)					Room temperature (25 °C)				
	Formulation					Formulation				
	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)	L	L (5 kGy)	L (10 kGy)	L (25 kGy)	L (50 kGy)
1	98.47 ± 1.26	95.47 ± 1.53	93.47 ± 1.22	91.47 ± 1.81	90.63 ± 1.14	88.92 ± 1.88	87.15 ± 1.19	85.11 ± 1.52	83.27 ± 1.59	82.33 ± 1.87
2	95.28 ± 1.17	94.13 ± 1.08	90.01 ± 1.12	91.22 ± 1.19	86.14 ± 1.22	86.92 ± 1.61	84.90 ± 1.43	83.75 ± 1.38	80.62 ± 1.90	77.26 ± 1.29
3	94.45 ± 1.42	93.27 ± 1.55	91.45 ± 1.71	90.45 ± 1.38	83.11 ± 1.29	77.17 ± 1.34	75.28 ± 1.41	74.43 ± 1.55	71.26 ± 1.82	68.46 ± 1.15

^aL formulation was dispersed in gel that is why the LJ results were the same with the L formulation

ties did not change after gamma irradiation. TLC analysis indicated that the R_F values of the DFNa-loaded formulations were significantly changed upon irradiation, which might indicate the degradation products. Those products might be analyzed with more sophisticated analytical methods, such as HPLC. pH of all formulations were significantly lowered upon irradiation process. Particle size distribution and encapsulation efficiency of L and N formulations were not affected by gamma irradiation. However, the phospholipid content of L formulations decreased which might be an indication for the damage of lipid bilayers. DFNa is entrapped in the liposomal core, that is why encapsulation efficiency was not affected while phospholipid content decreased. Release profiles of the L, N, LJ, and NJ formulations were altered, and the release rates were significantly increased with the irradiation process. The damage in lipid bilayers might facilitate the release of DFNa which is entrapped in the aqueous core in liposome/niosome and dispersed in lipogelosome/niogelosome formulations. Dynamic viscosity of the lipogelosome and niogelosome formulations were significantly lowered upon irradiation process. According to the sterility and pyrogen test results, there was no bacterial growth observed for all of the formulations. SAL of L, N, LJ, and NJ were found as 13.8, 15.2, 14.3, and 14.9 kGy, respectively, and all the SAL results were under the pharmacopeial obligatory dose rate which is 25 kGy. Stability test results indicated that the average particle size of the formulations did not change during the stability test conditions. Encapsulation efficiency of control and irradiated formulations were decreased both at room temperature and refrigerator temperature after 1 month. Gamma irradiation affects some of the physicochemical properties of DFNa-loaded formulations especially over 25 kGy. However, formulations can be sterilized safely by gamma irradiation method at predetermined SAL doses with minimum physical and chemical changes. The most important issue in radiation sterilization is to provide the optimum sterilization conditions. Further studies are needed to investigate the stability of the formulation upon gamma irradiation, like HPLC determinations, and improvement studies, like cryopreservation with different types of cryoprotectants.

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