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Influence of irradiation sterilization on poly(lactide-co-glycolide) microspheres containing anti-inflammatory drugs

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Abstract

γ-Irradiation is finding increasing use in the sterilization of pharmaceutical products. However, irradiation might also affect the performance of drug delivery systems. In this study, the influence of γ-irradiation on the physicochemical properties of two commonly used non-steroidal anti-inflammatory drugs (NSAIDs) [naproxen sodium (NS) and diclofenac sodium (DS)] was investigated. The drugs were incorporated in poly(lactide-co-glycolide) (PLGA, 50:50; molecular weight 34 000 or 88 000 Da) microspheres. The biodegradable microspheres were irradiated at doses of 5, 15, 25 kGy using a ^{60}Co source. Drug loading of irradiated and non-irradiated microspheres with both 34 000 and 88 000 Da polymers were essentially the same. A significant difference was noticed in the particle sizes of the irradiated as compared to the non-irradiated formulations. Notably, in release studies, the amount of active substance released from PLGA microspheres showed an increase with increasing irradiation dose. In DSC, the glass transition temperatures (T_g) of microspheres exhibited a slow increase with irradiation dose.

Keywords: Poly(lactide-glycolide) microspheres; γ-irradiation; Naproxen sodium; Diclofenac sodium

1. Introduction

Parenteral drug delivery systems based on PLGA polymers were intensively investigated in the last decades covering a wide variety of different drug substances [1]. Biodegradable microsphere formulation studies on two NSAIDs (DS and NS) have been previously reported from our laboratories [2,3]. The dispersion of NSAIDs into biodegradable polymer matrices has been accepted as a good approach for obtaining a therapeutic effect in a predetermined period of time, meanwhile minimizing side effects. The main requirement for an ideal intra-articular drug carrier system is that the system retains the active substance in the knee joints until it is taken up by the phagocytic cells. Incorporation of drugs within biodegradable polymeric particles has been shown to be effective in improving the retention of drugs within the joint cavity [3].

These systems have to meet the pharmacopoeial requirements of sterility, which is an important consideration often neglected in early development phases [4]. The chemical lability of active ingredients and polymeric matrix materials generally limits the strategies for obtaining an acceptable sterile product to aseptic processing and terminal sterilization using γ-irradiation.

Terminal sterilization of the parenteral delivery systems would be preferred from a microbiological safety point of view, since aseptic processing in a clean room environment under Good Manufacturing Practice (GMP) conditions is not only cost- and labor-intensive, but also inherently more risky with respect to microbial contamination of the finished product [4]. Since PLGA-based drug delivery systems are very sensitive to dry or moist heat, and ethylene oxide is not applicable due to its toxic residues, γ-irradiation currently remains the only accepted method for terminal sterilization of substances. Potential new techniques, e.g. low-temperature plasma sterilization are still under evaluation [1].

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Radiolytic degradation of incorporated drug and polymer matrix might be considered as the principal potential disadvantage of terminal γ-sterilization [5].

A number of studies have addressed the effects of γ-sterilization on biodegradable polymers [5–7]. It has been reported that biodegradable polyesters undergo chain scission and crosslinking after exposure to γ-rays. Usually, γ-irradiation of biodegradable PLGA or PLA reduces the molecular weight (MW) in a dose-dependent manner thus accelerating polymer degradation. Therefore, γ-irradiation might affect both the polymer decomposition and the release profile of incorporated drugs. It has also been reported that the impact of γ-sterilization on polymer degradation strongly depends on the irradiation dose [8]. Under the light of these data in recent literature [1–8] the goal of the present study was to evaluate the influence of different doses of γ-irradiation on the physicochemical properties and release of two anti-inflammatory drugs (NS and DS) incorporated in PLGA (50:50, MW, 34 000 and 88 000 Da) microspheres.

2. Experimental

2.1. Materials

The active substances were NS (Syntex Pharm., Switzerland) and DS (Novartis, Turkey). The matrix material was different grades (34 000 and 88 000 Da) of PLGA (50:50) (Medisorb, USA). Sodium oleate (SO), methanol, polyvinyl alcohol (PVA) were purchased from Merck (Germany). Methylene chloride was obtained from Quimon (Spain). The radiation source was a PX-γ-30 Issolodovatelj Irradiator (Hungary).

Table 1
The codes of microspheres which were non-irradiated and irradiated at different doses

Code	Active substance	PLGA (50:50) MW (Da)	Dose of irradiation (kGy)
A_{34}	NS	34 000	non-irradiated
$A_{34\text{-a}}$	NS	34 000	5
$A_{34\text{-b}}$	NS	34 000	15
$A_{34\text{-c}}$	NS	34 000	25
A_{88}	NS	88 000	non-irradiated
$A_{88\text{-a}}$	NS	88 000	5
$A_{88\text{-b}}$	NS	88 000	15
$A_{88\text{-c}}$	NS	88 000	25
B_{34}	DS	34 000	non-irradiated
$B_{34\text{-a}}$	DS	34 000	5
$B_{34\text{-b}}$	DS	34 000	15
$B_{34\text{-c}}$	DS	34 000	25
B_{88}	DS	88 000	non-irradiated
$B_{88\text{-a}}$	DS	88 000	5
$B_{88\text{-b}}$	DS	88 000	15
$B_{88\text{-c}}$	DS	88 000	25

2.2. Methods

2.2.1. Preparation of microspheres

The O/W single emulsion technique of solvent evaporation/solvent extraction was used for the preparation of NS and DS loaded microspheres [2,9–13]: 300 mg PLGA (34 000 or 88 000 Da) and 30 mg of NSAID (DS or NS) were dissolved in 6.5 mL methanol–methylene chloride (1.5:5). This solution was added to 100 mL of an aqueous solution of 500 mg PVA:SO (4:1) and stirred continuously at room temperature for 2 h until methylene chloride was evaporated. The microspheres obtained were washed four times with distilled water and dried in a vacuum oven at room temperature for 24 h.

2.2.2. Gamma sterilization of microspheres

Blank and NSAIDs incorporated microspheres were placed in vials, sealed under vacuum and γ-irradiated at ambient temperature (fix dose rate, 3.62 kGy/h; 5, 15 and 25 kGy doses) using ^{60}Co as the radiation source.

2.2.3. Determination of drug loading

Fifty milligram of the microspheres (non-irradiated or irradiated at different doses) were suspended in 10 mL of buffer (USP XXII pH 7.4 phosphate buffer for NS-PLGA microspheres; USP XXII pH 6.8 phosphate buffer for DS-PLGA) and placed in ultrasonic bath. Samples were filtered through 0.22 μm Millipore filters and the absorbance of the filtrate was measured (at 271 and 276 nm for NS and DS microspheres, respectively) to find out the active substance existing at the surface of the microspheres. The microspheres were then dried, weighed and dissolved by adding 5 mL of methylene chloride. Finally, polymer was precipitated by the addition of methanol (5 mL). The precipitate was removed by centrifugation for 15 min at 3000 rpm and the amount of entrapped active substance was determined in the clear supernatant.

2.2.4. Particle size analysis

Particle size and particle size distribution were analysed by dispersing the microspheres (non-irrradiated or irradiated) in an aqueous solution of Tween® 80 (0.1%). To disrupt microsphere aggregates that might be present, the samples were treated in an ultrasonic bath for 5 min. Laser light scattering determinations were made using a Malvern Mastersizer (Malvern Instruments, UK).

2.2.5. Surface morphology of microspheres

The irradiated and non-irradiated microspheres were evaluated for shape and surface characteristics by a

Table 2
Drug loading, particle size and the T_g values of microspheres which were non-irradiated and irradiated at different doses

Code	Drug loading (%)	Particle size 50% (μm)	T_g (°C)
A_{34}	12.00	9.00	36.22
$A_{34\text{-a}}$	11.85	19.93	37.46
$A_{34\text{-b}}$	12.06	51.74	38.84
$A_{34\text{-c}}$	12.13	53.05	39.25
A_{88}	10.00	5.00	41.17
$A_{88\text{-a}}$	10.24	22.53	41.25
$A_{88\text{-b}}$	12.13	38.02	41.73
$A_{88\text{-c}}$	10.31	41.83	41.84
B_{34}	12.70	29.39	39.20
$B_{34\text{-a}}$	12.76	25.39	40.03
$B_{34\text{-b}}$	12.68	39.01	40.75
$B_{34\text{-c}}$	12.56	54.60	41.30
B_{88}	16.10	5.77	41.17
$B_{88\text{-a}}$	16.21	40.82	42.20
$B_{88\text{-b}}$	16.06	76.17	43.75
$B_{88\text{-c}}$	15.94	96.62	44.40

scanning electron microscope (JEOL-SEM-ACID-10 Device in 80 KV). For this observation, microspheres were mounted on metal stubs with conductive silver paint and then sputtered with a 150 Å thick layer of gold in a Bio-Rad Apparatus.

2.2.6. In vitro drug release

In vitro release studies were carried out at 37 ± 0.5 °C, in pH 7.4 and pH 6.8 phosphate buffer for NS and DS incorporated microspheres, respectively. The microspheres (50 mg, non-irradiated or irradiated) were suspended in 25 mL of dissolution medium in a glass vial placed in a shaker bath (stroke, 50 cpm).

One milliliter aliquots were taken at predetermined time points (0, 1, 2, 12, 24, 36, 48, 60, 72, 84, 96, 120 h), filtered and replaced by equal volumes of the dissolution medium. The absorbance of released drugs was measured at 271 and 276 nm for NS and DS loaded microspheres, respectively.

2.2.7. Differential scanning calorimetry

Glass transition temperatures (T_g) of polymers and microspheres were recorded by a Dupont 951 Differential Scanning Calorimeter. Samples (6 mg) in sealed aluminium pans were heated from 0 to 100 °C twice, at a scanning rate of 10 °C/min. All glass transition temperatures reported correspond to the second measurement.

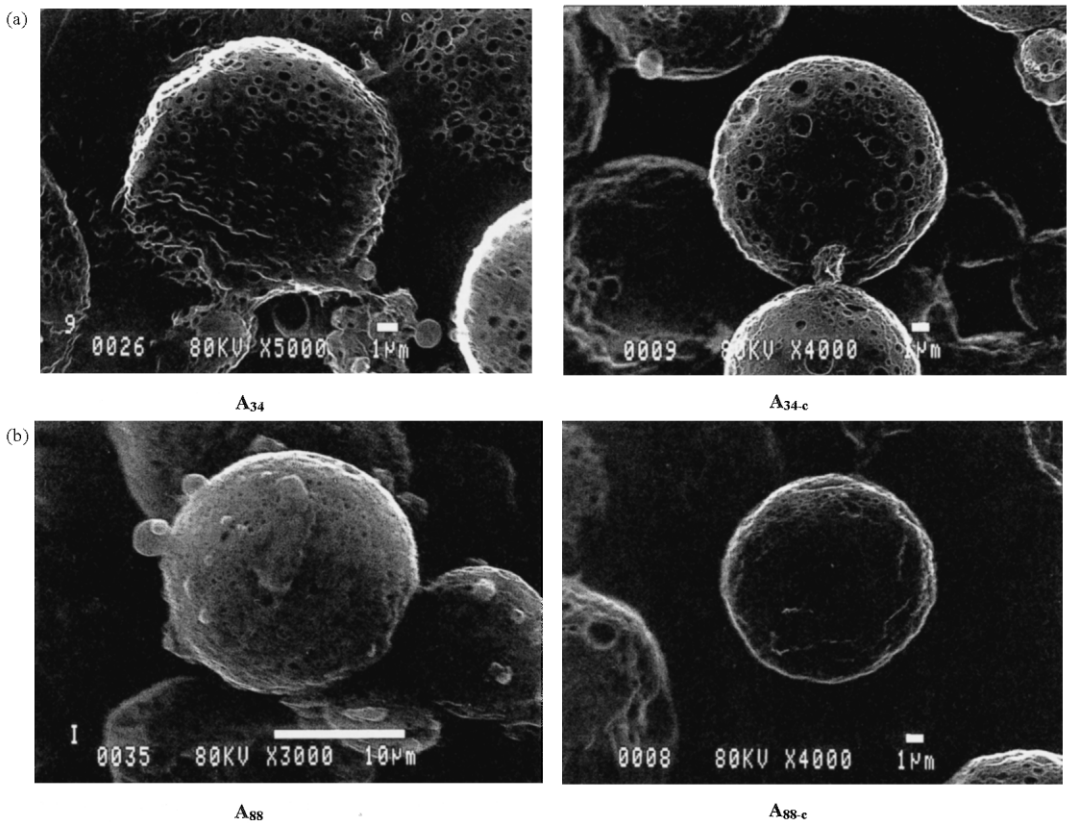

Fig. 1. NS containing PLGA microspheres. (a) MW of 34 000 Da; A_{34}: non-irradiated; $A_{34\text{-c}}$: 25 kGy irradiated. (b) MW of 88 000 Da; A_{88}: non-irradiated; $A_{88\text{-c}}$: 25 kGy irradiated.

3. Results and discussion

Drug solubility, internal morphology, solvent type, temperature, polymer composition, viscosity and drug loading are the important parameters which have been shown to influence the properties of microspheres during the manufacturing process [14]. In this study, a mixture of methanol and methylene chloride (1.5:5) was used as the organic phase to increase the solubility of the drug. Thus, the rate of precipitation of the polymer was faster in the droplet-water interface; resulting in minimal loss of drug into the outer aqueous phase and yielding homogenous and smaller particles [15]. Stirring time in the preparation of microspheres was optimized as 2 h, and allowed complete evaporation of methylene chloride from the medium. PVA and SO (4:1) were used as stabilizers. In previous studies, these substances and their ratio were reported to be critical in preserving the individuality of microspheres [16,17]. The codes of microspheres and the physicochemical characteristics of non-irradiated microspheres are presented in Tables 1 and 2, respectively.

The irradiation dose accepted to be satisfactory for sterilizing pharmaceutical products in accordance with good manufacturing practices [18–20] is 25 kGy. To check the influence of different doses of γ-irradiation on the physicochemical properties of NSAID loaded PLGA microspheres, 5 and 15 kGy was also examined. γ-irradiation doses applied to the microspheres are presented in Table 1.

γ-irradiation did not seem to have an effect in drug loading values, since no significant difference was observed between irradiated and non-irradiated microspheres derived from either 34 000 or 88 000 Da polymers (Table 2). Similar results have been reported in the literature previously [5].

Concerning the surface morphology; following the comparison of SEM photographs of irradiated and non-irradiated microspheres, the surface morphology of NS and DS loaded PLGA microspheres seemed to be affected by sterilization by γ-irradiation (Figs. 1 and 2). The changes in the morphology of the microspheres, were distinctly detectable by direct examination, but could not be equally well reproduced by the micrographs due to technical limitations of the system. After this explanation it is possible to state that the number of pores and their frequency of appearance was noticeable for non-irradiated microspheres while in irradiated microspheres the increase in the pore sizes with the similar frequency on the surface was observable to some extent (Figs. 1 and 2). Also some deformation in shape were appeared with the microspheres loaded with

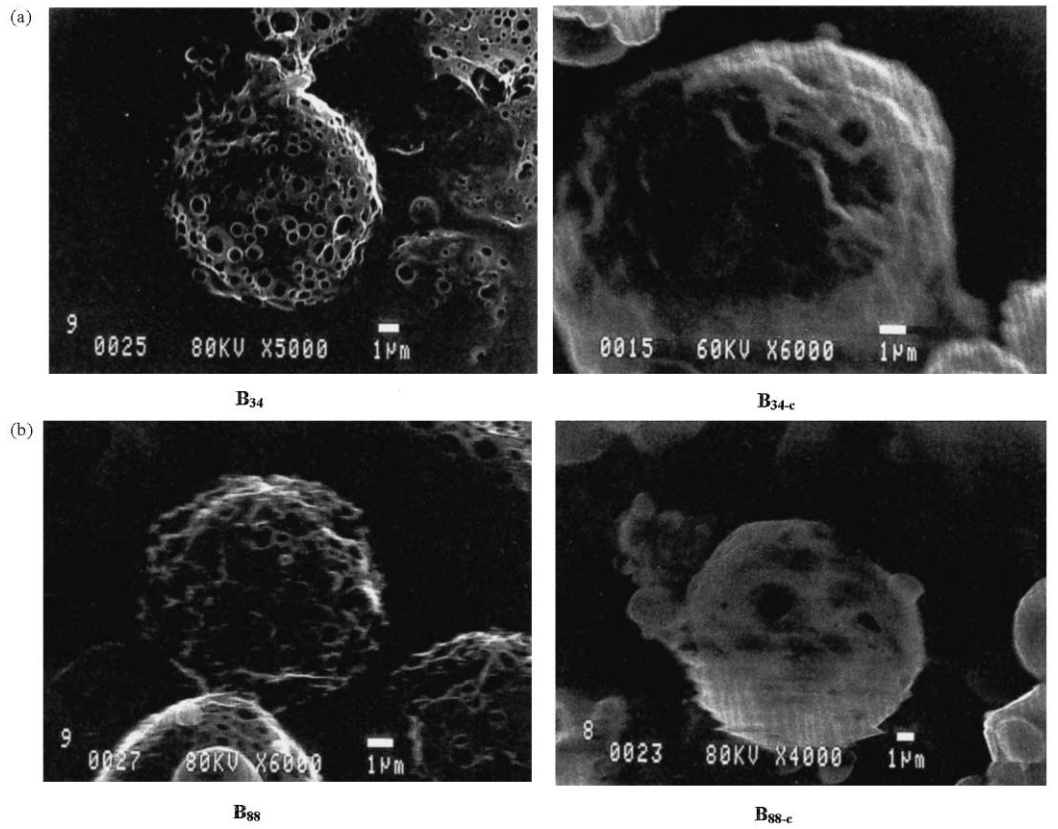

Fig. 2. DS containing PLGA microspheres. (a) MW of 34 000 Da; B_{34}: non-irradiated; $B_{34\text{-}c}$: 25 kGy irradiated. (b) MW of 88 000 Da; B_{88}: non-irradiated; $B_{88\text{-}c}$: 25 kGy irradiated.

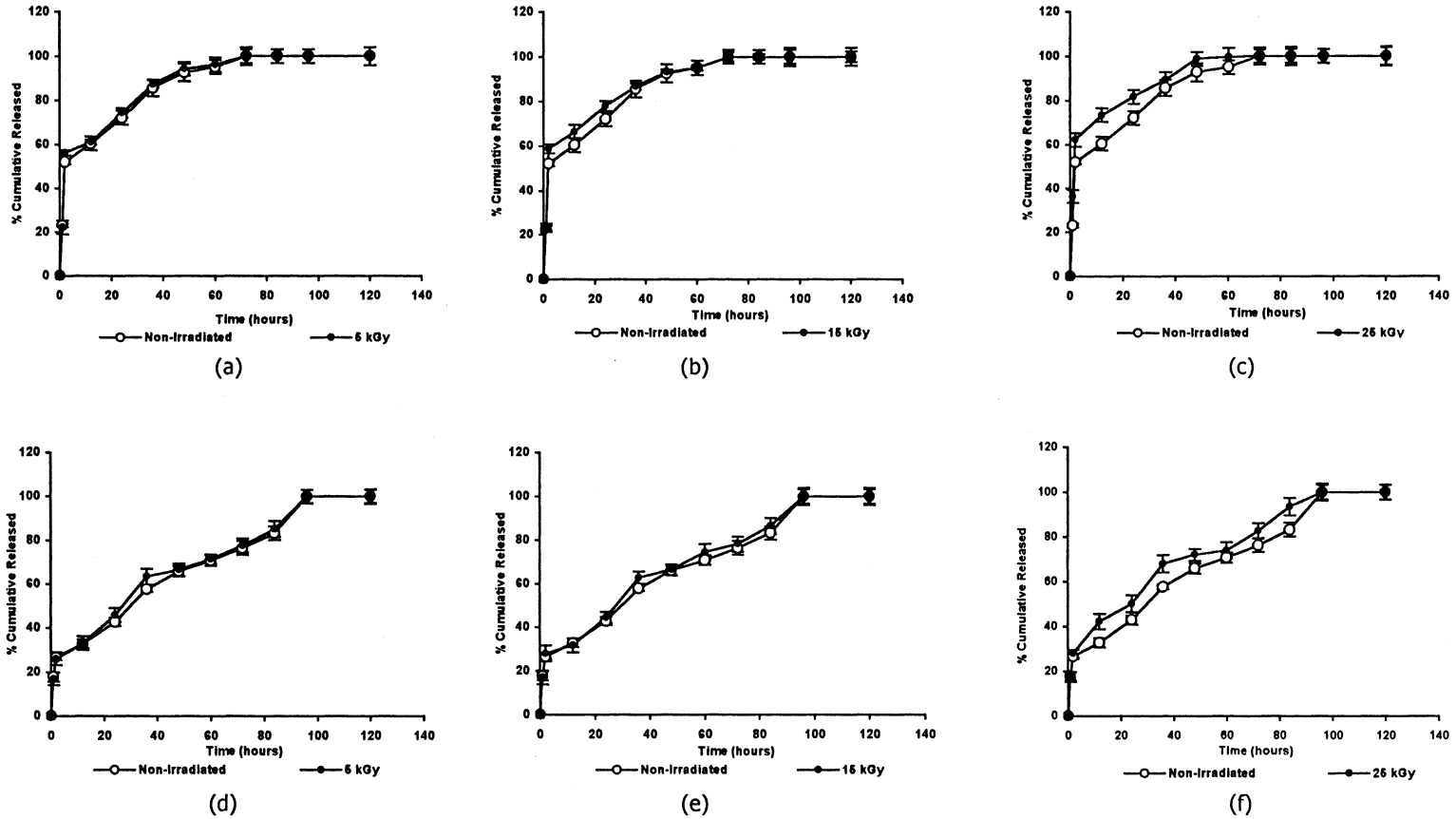

Fig. 3. NS loaded PLGA microspheres (Bars indicated SEM; $n = 6$). (a–c) MW of 34 000 Da; irradiated at 5, 15, 25 kGy, respectively. (d–f) MW of 88 000 Da; irradiated at 5, 15, 25 kGy, respectively.

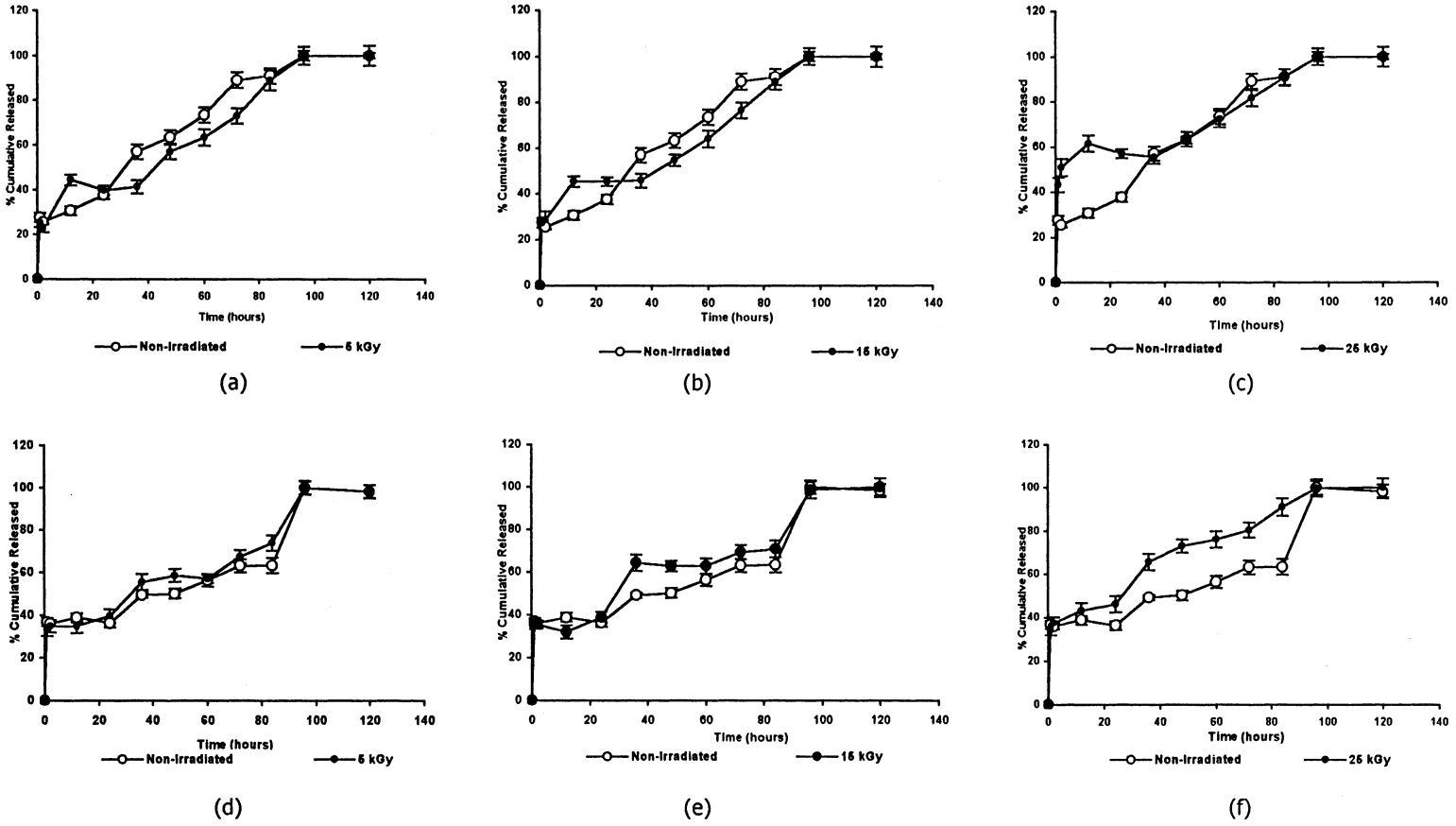

Fig. 4. DS loaded PLGA microspheres (Bars indicated SEM; $n = 6$). (a–c) MW of 34 000 Da; irradiated at 5, 15, 25 kGy, respectively. (d–f) MW of 88 000 Da, irradiated at 5, 15, 25 kGy, respectively.

DS and subjected to the highest irradiation dose (25 kGy) (Fig. 2). It is considered this might be an explanation of the noted increase in the release of DS from microspheres with increasing irradiation dose.

Granulometric analysis of the NSAID-loaded microspheres performed on biopolymers of either MW showed that there was a marked increase in particle size which appeared to relate to irradiation dose. Hartas et al. [7] have suggested that γ-irradiation may cause some deleterious changes in the mean diameters of microspheres as well as in the molecular weight and morphology of the polymer which in turn may control the biodegradation and drug release characteristics. With the NS- and DS-loaded microspheres (MW 88 000 Da), the mean diameters of the microspheres showed an interesting change: They were measured to be 5 and 5.77 μm before irradiation and increased up to 41.83 and 96.62 μm, respectively, for the same formulations following irradiation at 25 kGy (Table 2). Montanari et al. [21] observed a very similar situation in PLGA microspheres, in which 70% of the particles possessed diameters of 15 μm before irradiation while the mean diameters of 80% of particles increased up to 25 μm, following the irradiation at 25 kGy dose. The authors reported that this phenomenon could be attributed to the formation of aggregates.

Concerning the released amounts of NS and DS from PLGA microspheres following the sterilization process, a dose dependent acceleration was noticed (Figs. 3 and 4). The drug release patterns of the irradiated and non-irradiated microspheres were compared by Randomized Complete Block Design statistics (in NS microspheres $F = 9.76$, 10.94 for 34 000 and 88 000 Da MW, respectively; in DS microspheres $F = 7.08$, 3.47 for 34 000 and 88 000 Da MW, respectively; $P < 0.05$). Release patterns of the irradiated and non-irradiated microspheres appeared to be similar, while the released percentages showed changes as stated below. Regarding NS microspheres: (i) for 34 000 Da MW, the amount released by the end of 24 h was about 72.16% for non-irradiated microspheres, whereas the corresponding values were 74.46, 78.12, 81.76% for 5, 15, 25 kGy irradiated formulations, respectively (Fig. 3a–c); (ii) for 88 000 Da MW, released NS at the end of the same period from the non-irradiated microspheres was 42.88% whereas it was recorded as 45.96, 44.63, 50.16% for 5, 15, 25 kGy irradiated formulations, respectively (Fig. 3d–f). Similarly, in DS loaded PLGA microspheres: for MW 34 000 Da, released DS from non-irradiated microspheres was 37.72% at the end of 24 h (for MW 88 000 Da, 36.41%) whereas it was obtained as 39.69, 45.32, 56.92% (for MW 88 000 Da, 39.75, 38.89, 46.23%) from 5, 15, 25 kGy irradiated formulations, respectively (Fig. 4a–f). In accordance with our results, some authors reported that in vitro release from PLGA microspheres might be affected by γ-sterilization [4,22]. Spenlehauer et al. [22] stated that cisplatin-loaded PLA or PLGA microspheres irradiated at 37.7 or 28.4 kGy doses showed a sharp decrease in polymer molecular weight. Thus the drug was released much faster; in vitro release decreased from 60 to 8 days. Volland et al. [4] investigated the influence of different irradiation doses (6.9, 15, 27.7 and 34.8 kGy) on captopril-loaded microspheres and obtained a dose dependent acceleration in the in vitro release of the active substance.

In the light of the DSC results, it was observed that the T_g of irradiated microspheres gradually increased with increasing irradiation dose (Table 2). As the change in DSC findings were not noteworthy, it seemed hard to correlate these data with the increased in vitro release from the irradiated microspheres.

This is a preliminary study to evaluate the influence of γ-irradiation on the physicochemical properties and release of two anti-inflammatory drugs loaded in PLGA microspheres. The investigation may be extended to include additional experimental data on physicochemical properties of polymers as molecular weight and polydispersity index. These aspects of the system will be taken up in future studies.

In conclusion, although γ-irradiation appears to be a potential sterilization procedure for parenteral drug delivery systems such as microspheres and implants based on biodegradable polyesters, it should be carefully evaluated and used with caution since irradiation sterilization might cause changes in the properties of drug delivery formulations.

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