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Study of gamma-irradiation effects on aciclovir poly(D,L-lactic-co-glycolic) acid microspheres for intravitreal administration

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Abstract

Gamma-irradiation effects on aciclovir poly(D,L-lactic-co-glycolic) acid (PLGA) microspheres, with gelatin as additive, were studied. Microspheres with a 2:2:10 aciclovir:gelatin:polymer ratio were prepared by the solvent evaporation method and sterilised by γ-irradiation at a dose of 25 kGy. Loading efficiency, morphology (particle size analysis, scanning electron microscopy (SEM)), physical chemistry (infrared (IR) absorption spectrophotometry, differential scanning calorimetry (DSC), X-ray diffraction and gel permeation chromatography (GPC)) and in vitro release assays for 73 days were performed to evaluate the sterilisation effect on microsphere characteristics. After γ-irradiation, no surface changes were observed by SEM. Microparticle mean diameter and aciclovir loading efficiency were not affected by γ-ray exposition. IR spectroscopy, DSC and X-ray diffraction showed no modification of the bulk properties of the microspheres or their components. The controlled release profiles of aciclovir-loaded microspheres for 73 days were not altered upon exposure to γ-irradiation. GPC measurements showed a decrease in molecular weight of the polymer. The sterilisation method is adequate because microspheres underwent no change after exposition to γ-irradiation. These favourable properties of the aciclovir-loaded microspheres make them a suitable system for the intravitreal treatment of herpes virus infections, in an animal model.

Keywords: Aciclovir; Gelatin; PLGA microspheres; Intraocular; Sterilisation

1. Introduction

Some ocular pathologies such as herpes simplex virus retinitis and acute retinal necrosis are usually treated by administering aciclovir. It has been demonstrated that intravitreal administration of aciclovir is more effective than intravenous administration [1]. Nevertheless, due to its short vitreous half-life, it is necessary to administer several doses to maintain therapeutic drug concentrations with the inherent risks of successive intraocular injections (vitreous haemorrhage, endophthalmitis, retinal detachment and cataract). These inconveniences could be overcome by

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using drug delivery systems able to promote prolonged drug release into the vitreous cavity such as biodegradable microspheres.

Poly(D,L-lactic-co-glycolic) acid (PLGA) is a common, biodegradable polymer currently used in injectable drug delivery systems and especially for controlled release of drugs to be injected directly into the eye. PLGA has been widely used in drug formulations such as microspheres. In previous studies carried out by the authors, aciclovir–PLGA microspheres were prepared by the solvent evaporation method with several additives to identify a potential formulation, which would provide controlled and predictable release kinetics including a minimal burst effect and a lasting release. Best results were obtained when gelatin was added to the external phase of the emulsion [2]. Further, this formulation was optimised according to a factorial design, to reduce, as much as possible, the quantity of microspheres to be administered. The best formulation according to the selected design was prepared with a 2:2:10 aciclovir:gelatin:-polymer ratio, which released aciclovir at a constant rate for 63 days, resulting potentially useful for the treatment of ocular pathologies caused by herpes simplex and varicella zoster viruses in single intravitreal doses of 0.48 and 4.8 mg of microspheres, respectively [3].

Microspheres intended for intravitreal administration, as with other injectable delivery systems, have to meet the pharmacopoeia requirements of sterility. It is well known that most sterilisation techniques, such as sterilisation by steam and dry heat cannot be used for biodegradable aliphatic polyesters, such as PLGA and polylactic acid (PLA), since they alter the physical chemistry of the polymer. Chemical sterilisation with ethylene oxide causes serious toxicological problems due to residues of the sterilising agent [4]. Moreover, the stability of a drug incorporated into the polymer matrix can also be altered [5].

PLGA is highly sensitive to moisture and high temperatures. Chemical lability of the polymeric matrix material, as well as some active constituents limit the strategies to obtain an acceptable sterile product to aseptic processing and terminal sterilisation using high-energy irradiation. Terminal sterilisation of injectable drug delivery systems would be preferred from a microbiological point of view, since aseptic processing in a clean room environment under good manufacturing practice conditions is not very cost and labour intensive, but has inherently more risk with respect to microbiological contamination of the finished product. Irradiation treatment, especially γ-irradiation, is successfully employed for the sterilisation of thermolabile medical devices, such as catheters or syringes and polymeric biodegradable delivery systems. Furthermore, it is also possible to sterilise pharmaceutically active substances with high-energy radiation.

In the pharmaceutical field, one of the applications of ionising radiation is the final sterilisation of biodegradable PLGA microspheres intended for parenteral use [6]. The advantages of sterilisation by irradiation include high penetrating power, low chemical reactivity, low measurable residues, small temperature rise and fewer variables to control. Sterilisation of a product must produce a safety level, sterility assurance level (SAL) of 10^{-6}. A minimum absorbed dose of 25 kGy is regarded as adequate for the purpose of sterilising pharmaceutical products without providing any biological validation [7].

Gamma-radiation as a form of electromagnetic radiation, characterised by high penetration at a very low dose rate, can modify the performance of irradiated drug delivery systems, prolonging the peroxidative radiolitic mechanism due to the exposure time [8]. The polymer degradation products generated can significantly alter the aqueous microenvironmental conditions, e.g. H^+ concentration within the system. In release assays, lactic and glycolic acids produced by degradation of PLGA polymers can lead to a significantly decrease of pH values at the centre of dosage forms [9]. As PLGA degradation is catalysed by protons, this micropH decrease can lead to autocatalytic effects and, thus, accelerate polymer degradation [10,11]. Nevertheless, the absorption of hydroxide ions from the release medium into the system and/or the diffusion of monomeric and/or oligomeric acids out of the system can be rapid enough to prevent acidic microenvironments and, thus, suppress any autocatalytic effect [12,13]. All these events take place as a function of the size and porosity of the device. Furthermore, polymer degradation products can crystallise within the dosage form [14,15], modifying porosities. Depending on their solubility and the microenvironmental conditions, these degradation products subsequently dissolve

and diffuse out of the device. Gamma-irradiation of bioresorbable polyesters induces dose-dependent chain scission as well as molecular weight reduction [16], with the reduction a function of the polymer molecular weight and, thus accelerating degradation of the polyester. The degradation rate of polymeric biomaterials due to γ-irradiation is linked to radical formation [17]. In order to prevent radical-induced degradation, antioxidants are often added to pharmaceutical preparations. Pharmacopoeias propose α-tocopherol and ascorbic acid esterified with palmitin- or stearic-acid as protecting agents for lipophilic materials. Sterilisation under nitrogen monoxide has also been used for this purpose.

As commented above, the effects of γ-irradiation on PLGA and loaded microspheres have been discussed by several authors, and different results have been reported, depending on the active component [5,18–21]. That makes it necessary to study the effects of γ-irradiation on new formulations such as microspheres intended for intravitreal administration because local toxicity is related to particular properties that can be affected by sterilisation.

In this study, the effects of γ-irradiation at a dose of 25 kGy on microspheres made of PLGA and loaded with aciclovir, which have shown an optimum release [3], were evaluated in order to continue with in vivo studies. The influence of irradiation on the selected formulation was investigated using various different techniques: scanning electron microscopy (SEM), infrared (IR) spectroscopy, differential scanning calorimetry (DSC), X-ray diffraction and gel permeation chromatography (GPC). Aciclovir release profiles of sterilised and non-sterilised microspheres were also compared.

2. Materials and methods

2.1. Materials

Aciclovir (acicloguanosine, 9[2-(hydroxyethoxy)-methyl]-guanine) was purchased from Reig Farma, S.A. (Spain). Poly(lactic-co-glycolic acid) 50:50, Resomer® RG 502 (PLGA), Mw 15000 Da, inherent viscosity 0.2 dl g^{-1} was provided by Boehringer Ingelheim Chemicals Division (Germany). Polyvinyl alcohol (PVA, Mw 72000 Da) and gelatin (type A, 100–120 bloom) were supplied by Fluka Chemie AG (Germany) and Merck (Spain), respectively.

Tetrahydrofuran, HPLC grade, was received from Ryedel de Haen (Spain). Methylene chloride (CH_2Cl_2) and sodium hydroxide solution, analytical grade, were obtained from Merck (Spain). Distilled and deionised water (Millipore, MA, USA) was used in preparation of buffers and solutions.

2.2. Preparation of biodegradable microspheres

Aciclovir-loaded PLGA microspheres were prepared by the solvent evaporation method based on an oil-in-water (o/w) emulsion [2]. The starting quantities of drug and polymer were 80 and 400 mg, respectively. The quantity of gelatin used in microsphere preparation was 80 mg.

Briefly, the organic phase was prepared by dissolving the polymer in 1 ml CH_2Cl_2 using a vortex mixer (IKA Labortechnik, Germany) and then dispersing aciclovir in the polymer solution. The aqueous phase consisted of a PVA solution (0.1%) in which gelatin had been dissolved. This solution was prepared as follows: gelatin was dispersed in approximately 10 ml of 0.1% PVA, allowing the gelatin particles to swell, and afterwards the dispersion was heated at 50 °C under magnetic stirring. After cooling, this solution was added to 0.1% PVA to complete the volume to100 ml. The organic phase containing the drug suspension was slowly added to the aqueous phase to form an emulsion, which was continuously stirred for 3 h at room temperature to allow complete evaporation of the organic solvent.

Upon evaporation of dichloromethane, microspheres were vacuum-filtered through a 5-μm filter, washed three times with water and lyophilised (Flexy-Dry™, FTS Systems, USA). Lyophilised microspheres were kept in a desiccator until use.

2.3. Gamma-irradiation of aciclovir microspheres

Samples were conditioned according to the process described by Hausberger et al. [18]. Seven different batches of microspheres were weighted (100 mg) and transferred to 5-ml glass aluminium sealed cap vials. Vials were labelled and packed surrounded with dry ice into a polyurethane container, assuring a low temperature during the irradiation process. Although

γ-irradiation causes only a minimal temperature rise, keeping low temperature avoids a possible acceleration of PLGA hydrolytic degradation. Samples were shipped and irradiated with Co-60 at the Gamma-Sterilisation Unit of Aragogamma S.A. (Spain). Following the USP recommendations, an effective sterilising dose of 25 kGy (commonly known as the industrial overkill) was used.

2.4. Determination of aciclovir loading efficiency

Microspheres (10 mg) were dissolved in dichloromethane (1 ml). Aciclovir was extracted from the CH_2Cl_2 solution three times with 9 ml of sodium hydroxide solution (10^{-4} M) and centrifuged (Eba 12R, Hettich, Germany) at $6000 \times g$ for 5 min. Top layers were filtered through a 0.45-μm syringe filter (Tracer, Spain). Aciclovir was assayed spectrophotometrically (DU-6, Beckman, OH, USA) at 254 nm from the extracted aqueous solutions. The total amount of aciclovir was calculated from aliquots of each extract, in triplicate for each batch of γ-irradiated and non-irradiated microspheres.

Data obtained spectrophotometrically were confirmed by HPLC according to the technique described by Boulieu et al. [22]. A Gilson HPLC system (Gilson Medical Electronics, Middleton, WI, USA) was equipped with a model 305 piston pump, a model 118 UV detector and a 712 system controller software. A Hypersil ODS (150 × 4.6 mm, 3 μm) column (Tecknocroma, S. Coop., Barcelona, Spain) was used with a mobile phase consisting of H_2KPO_4 0.02 mol l^{-1}, pH 3.5. The flow rate was 1.5 ml min^{-1}, and the detection wavelength was set at 254 nm. The retention time was 9.8 min.

2.5. Morphological characterisation and size distribution

Microsphere samples were observed by light microscopy. Surface morphology of microspheres at different stages of the in vitro release assays was investigated by SEM (Jeol, JSM-6400, Japan). Samples were dried and gold sputter-coated before observation by SEM at 20 kV.

Particle size analysis was performed in a Galai Cis-1 computerised inspection system (Galai Production Ltd., Israel) with laser diffraction optics. Size measurement of the Cis-1 version ranged from 0.5 to 150 μm. Samples of microspheres were suspended in distilled water and analysed while gently stirring. Results are expressed as volume–density mean diameter.

2.6. Injectability

Approximately 10 mg of aciclovir-loaded microspheres were suspended in 1 ml saline solution. Two millilitre syringes, fitted with needles of different inner diameter, containing the microsphere suspension were placed in an Instron 4501 instrument (Instron, MA, USA) to measure ejection force.

2.7. Infrared absorption spectrophotometry (IR)

IR spectra were recorded on a Perkin–Elmer system 2000 FT-IR IR spectrophotometer (Perkin–Elmer, UK). Scans for samples were recorded at a resolution of 2 cm^{-1} over the wave number region 400–4000 cm^{-1}. Samples were mixed with KBr grain spectroscopy grade and pressed into a disc by compaction. IR spectra before and after sterilisation were recorded for aciclovir, PLGA and microspheres. The weight ratio KBr:powder was about 100:1.

2.8. Differential scanning calorimetry (DSC)

DSC analysis was performed with a Mettler 820 DSC analyser (Mettler Toledo, Switzerland). Indium standards were used for system calibration. Sterilised and non-sterilised microsphere samples (5–10 mg) were sealed in aluminium pans and heated under inert atmosphere (40 ml min^{-1} of nitrogen) against an empty pan as reference. Scans were obtained under heating conditions from 25 to 300 °C and heating rate of 10 °C min^{-1}. Data were processed by the system software and glass transition temperatures (T_g) and crystalline melting points (T_m) were identified.

2.9. X-ray diffraction

X-ray diffraction patterns were measured in an automatic powder Philips X-PERT MPC diffractometer (CAI, DRX, UCM) combined with a high temperature chamber (Anton Paar HTK 10) with a Pt heating filament, Ni-filtered Cu–K_α irradiation

($\lambda = 1.54056$ Å), a 2θ interval configuration, angle range 3–60°, scan step size 0.04 ° and time per step 1 s. Under these conditions, samples of aciclovir, polymer, non-loaded microspheres and γ-irradiated and non-irradiated aciclovir-loaded microspheres were assayed.

2.10. In vitro release studies

Sterilised and non-sterilised aciclovir-loaded microspheres (10 mg) were suspended in 3 ml of isotonic phosphate buffer saline (PBS) pH 7.4 (sink conditions) and placed in a water shaker bath (NE-5, Clifton, UK) at 37 °C with constant agitation (100 strokes per min). At fixed time intervals, PBS was removed with a syringe, filtered through 0.45-μm filters and aciclovir concentration was measured spectrophotometrically at 251 nm (no microsphere component interfered with aciclovir). The same volume of fresh medium was replaced to continue the release study. This assay was performed in duplicate for each batch of microspheres (seven batches).

After the release assay, microspheres were dried for 2 days in a vacuum desiccator and residual aciclovir was extracted and analysed.

2.11. Gel permeation chromatography (GPC)

Microparticles were dissolved in tetrahydrofuran (5 mg ml^{-1}). After filtration (PTFE filter, pore size 0.45 μm, Tracer), 10 μl of the solution were injected in a Styragel HR 4E column (7.8 × 300 mm, Waters, MA, USA). All measurements were performed at a flow rate of 1 ml min^{-1} at working temperature with a Waters 1525 binary HPLC pump. The refractive indexes were measured using a 2414 refractive index detector (Waters). Molecular weights were calculated by the system calibration software. Calibration was performed using narrow polystyrene reference materials of Mw: 114,000, 43,700, 18,600, 9650, 6520 and 2950 Da (Waters, Polymer Standard Service, Germany). Evaluation was done according to a cubic universal calibration curve (Waters). Molecular weights are expressed as weight-average molecular weights (*Mw*) and number-average molecular weights (*Mn*). Samples of γ-irradiated and non-irradiated microspheres at different stages of the in vitro release assay were analysed.

3. Results and discussion

Aciclovir loading efficiency, physical chemistry as well as release profiles of sterilised and non-sterilised microspheres were studied.

Aciclovir mean loading efficiency was not affected by the sterilisation process ($P < 0.05$), with values $70.86 \pm 2.15\%$ and $70.98 \pm 2.36\%$, for irradiated and non-irradiated microspheres, respectively. Although data obtained by HPLC before and after irradiation did not show significant differences ($P < 0.05$), further studies would be necessary to confirm the absence of bond scission of the drug molecule in the microspheres after γ-irradiation exposure.

Mean diameters of sterilised (45.47 ± 13.36 μm) and non-sterilised (46.38 ± 12.79 μm) microspheres were not significantly different. These results showed that no morphological change due to temperature rise, e.g. particle fusion, took place after gamma-irradiation treatment because samples were protected with dry ice during irradiation exposure.

Injectability of microspheres is an important criterion so that microspheres can be administered through a needle of minimum inner diameter for a successful intravitreal injection. Application of a maximum ejection force of 12 Newtons over 10 s can be considered as a suitable development criterion. Tests were performed on a suspension of microspheres in saline solution with no dispersability modifier agent. Suspensions of sterilised microspheres were injected through different needle diameters (27G, 25G and 21G). Data (12.5, 8.4 and 5.5 N, respectively) indicated neither partial nor complete blockage of the suspension flow. These results suggest that because of the small particle diameter no injection difficulties were developed. Thus, they are suitable for intraocular injection through a 27G needle. Furthermore, the use of substances such as hydroxypropylmethylcellulose or hyaluronic acid to improve injectability would not be necessary [23].

The IR absorption spectra obtained for aciclovir, PLGA, non-loaded microspheres, and aciclovir-loaded microspheres did not change due to the sterilisation process (Fig. 1). FT-IR spectrum of sterilised PLGA (1) showed characteristic absorption bands in the 1300–1500 cm^{-1} region: a C–H bending vibration of methyl group at 1397 and 1456 cm^{-1}, and an additional C–H vibration of methylene group at

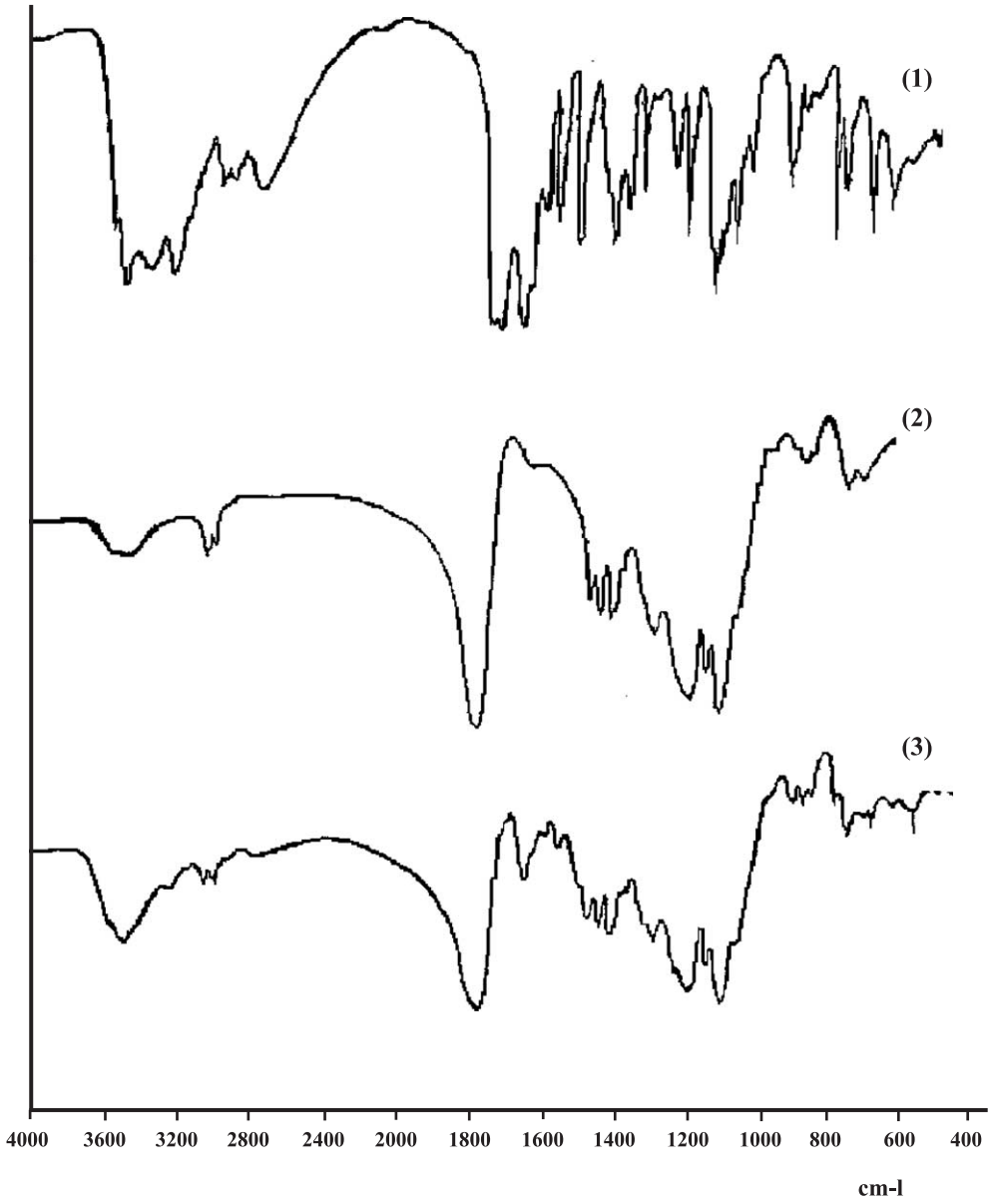

Fig. 1. IR spectra for PLGA (1), aciclovir (2) and γ-irradiated aciclovir-loaded microspheres (3) (non-irradiated microspheres not shown). Percentage transmittance scale is arbitrarily applied.

1426 cm^{-1}. FT-IR spectrum of γ-irradiated aciclovir (2) showed the same absorption bands as non-irradiated aciclovir (non-irradiated aciclovir data not shown). IR spectra of sterilised (3) and non-sterilised microspheres (non-sterilised microsphere spectrum overlapped with the sterilised microsphere spectrum) exhibited a transmission pattern with the PLGA and aciclovir absorption bands.

Samples of polymer, non-loaded microspheres and aciclovir-loaded microspheres were analysed by DSC before and after sterilisation. Non-loaded microspheres (II) showed the same glass transition tempe-

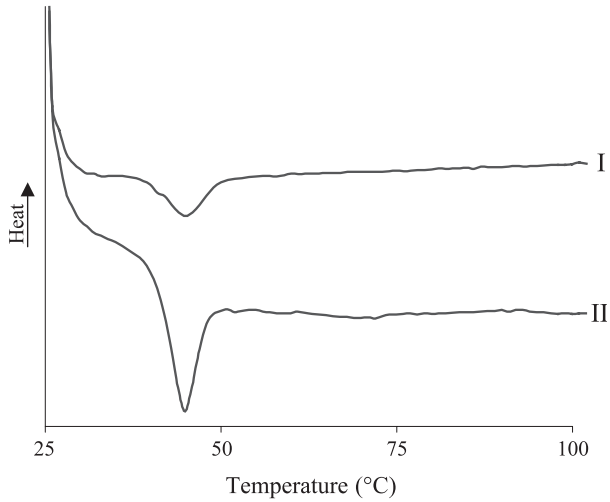

Fig. 2. DSC thermograms for PLGA (I) and non-loaded microspheres (II).

rature as PLGA (I) (T_g = 44.83 °C) (Fig. 2), revealing that production process did not affect the copolymer structure. Nevertheless, gamma-irradiation decreased T_g values of polymer (41.12 °C) and non-loaded microspheres (37.17 °C). It is known that relatively low glass transition temperatures could favour subsequent reactions of free radicals due to a higher mobility of the polymer chains [5]. For its part, the DSC thermogram of aciclovir showed a broad endotherm in the range 75–125 °C corresponding to a loss of residual water, a narrow melting endotherm at 255.33 °C and a small endothermic peak around 175 °C [2]. DSC thermogram melting endotherms at 255.33 °C of sterilised and non-sterilised aciclovir-loaded microspheres (Fig. 3) were similar. In both cases, a broad endotherm was observed in the 75–100 °C range, which corresponded to a loss of residual water. The aciclovir melting endotherm remained located at practically the same temperature for non-sterilised and sterilised microspheres (T_m = 243.67 and 240.67 °C, respectively), but decreased with respect to aciclovir raw material, probably due to an interaction between the formulation components. T_g value (44.67 °C for non-sterilised) very slightly increased

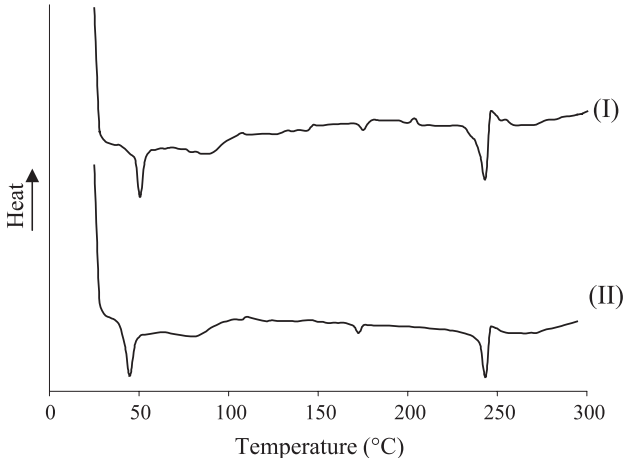

Fig. 3. DSC thermograms for sterilised (I) and non-sterilised (II) aciclovir-loaded microspheres.

for sterilised aciclovir microspheres (46.52 °C). This shift of PLGA T_g to higher temperatures can be attributed to a lower mobility of the polymer chains probably due to aciclovir. The small endothermic peak around 175 °C for aciclovir raw material was also observed (173 °C for non-irradiated and 177 °C for γ-irradiated microparticles) and might be related to the fusion of a morphous form of aciclovir. The sterilised microspheres showed an exothermic peak at 204.17 °C, probably related to the heating process during the sample calorimetric assay.

The strongest maxima of intensity in the X-ray diffraction pattern for aciclovir were obtained at 6.94°, 10.46°, 23.82°, 26.06° and 29.18°. On the contrary, the polymer pattern showed no maxima confirming its amorphous state. The microencapsulation process did not modify the polymer characteristics as can be observed in non-loaded microsphere X-ray diffraction patterns. Aciclovir-loaded microspheres (irradiated and non-irradiated) showed the maxima of crystalline aciclovir (6.98°, 10.5°, 23.94°, 26.14° and 29.26°) superposed on the polymer pattern. These data confirmed the DSC results indicating that no specific interactions between aciclovir and PLGA occurred, although there is no clear evidence about chemical interactions (Fig. 4). Aciclovir is incorporated in the microspheres in the crystalline state, and the evaporation solvent process does not modify the crystalline halos of the drug.

Fig. 5 shows SEM photographs of sterilised aciclovir-loaded PLGA microspheres at different stages ($t = 0$, 8, 28, 50 and 73 days) of the release assay. At time $t = 0$ (A) (before drug release) microparticle shapes were spherical and possessed a smooth surface with no macropores (size >100 nm). No drug crystals appeared on their surface. On eighth day (B), although sterilised microspheres were agglomerated, surface morphology remained almost unchanged. On 28th day (C) a loss of structure was observed, which was in progress until day 50 (D), and on the 73rd day (E) only remnants of microsphere walls (“ghost-like”

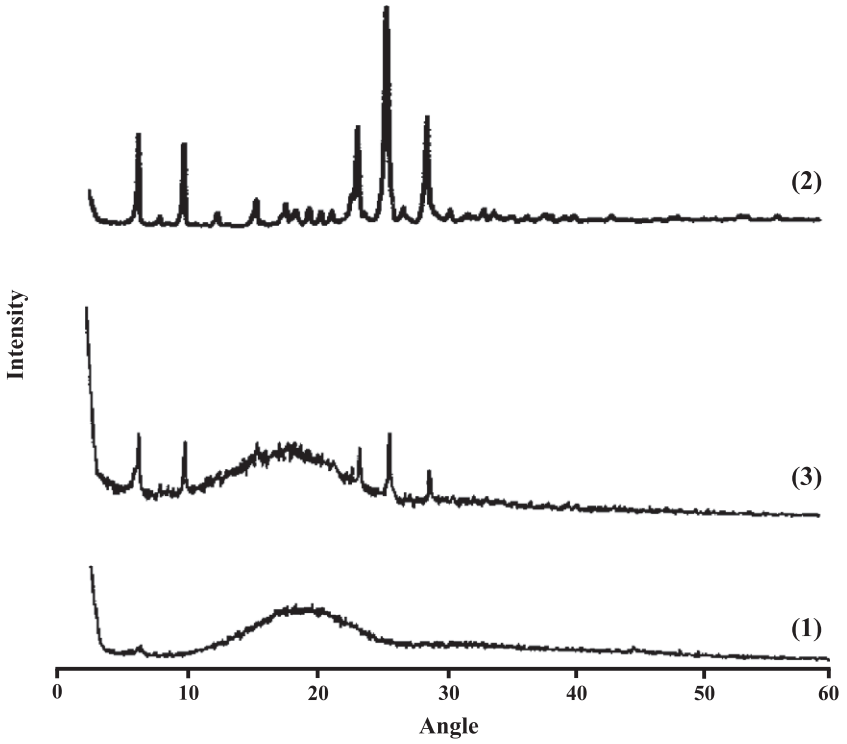

Fig. 4. X-ray scattered intensity as a function of the diffraction angle 2θ. Intensity scaling is arbitrarily applied to the curves. Curves obtained for polymer (1), aciclovir (2) and γ-irradiated microspheres (3).

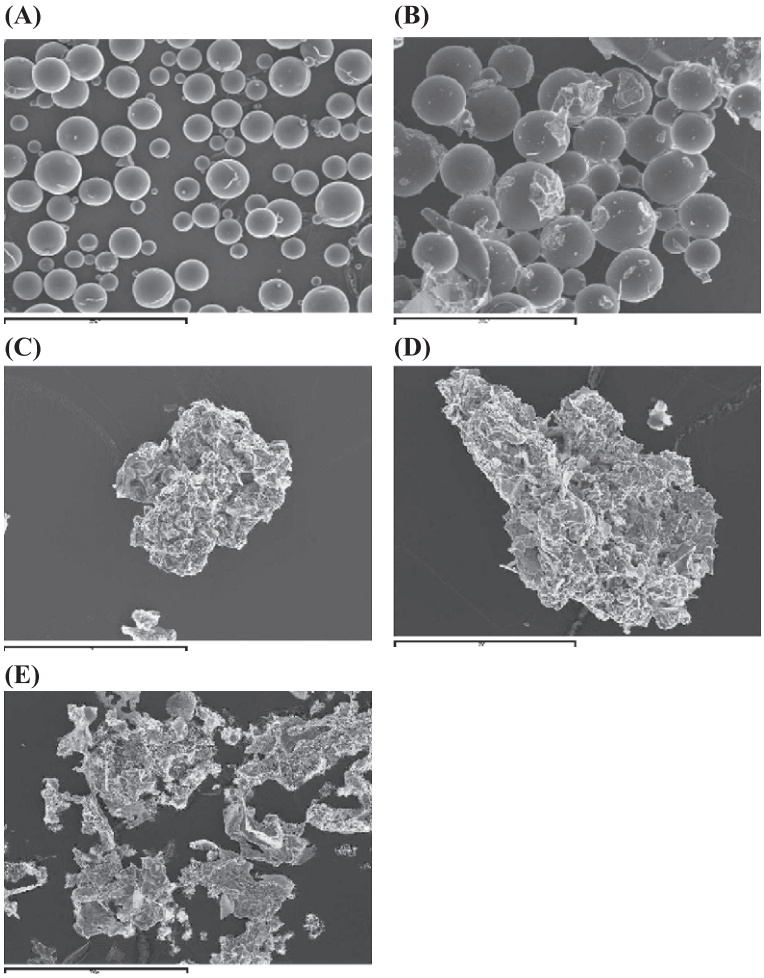

Fig. 5. SEM photographs of aciclovir-loaded microspheres surfaces upon exposure to phosphate buffer pH 7.4 at 37 °C, $t=0$ (A), 8 (B), 28 (C), 50 (D) and 73 (E) days (scale 200 μm). Only sterilised microparticles are shown, morphologies of non-sterilised microparticles are very similar.

structures) remained. Surfaces of non-sterilised microparticles were similar (data not shown).

PLGA microspheres have demonstrated, in some cases, sensitivity to γ-irradiation increasing the in vitro drug release rate. The percentage of cumulative aciclovir released in vitro from seven batches of non-irradiated and γ-irradiated microparticles was examined in PBS buffer for up to 2 months (Fig. 6). Similarity factor (f_2) [24,25] was calculated to compare release profiles of aciclovir from microspheres before and after sterilisation. Conceptually, f_2 is a measure of the similarity in the dissolution percentage between two profiles (a value ranging between 50 and 100 indicates similarity between curves). Table 1 lists f_2 values obtained, in the range 92.78–99.81, so both curves can be considered similar, results which are in agreement with those previously obtained for ganciclovir [26].

Release profiles from sterilised and non-sterilised aciclovir microspheres were biphasic and showed a low initial burst followed by a zero-order phase. Drug was released from microspheres for 73 days in both cases. Aciclovir release followed a zero-order kinetic from 1 to 63 days with a mean release constant of 1.735 ± 0.089 μg per day per mg (non-sterilised microspheres) ($r=0.997$) and 1.733 ± 0.093 μg per day per mg (sterilised microspheres) ($r=0.998$). These values were compared with the Kurskal–Wallis

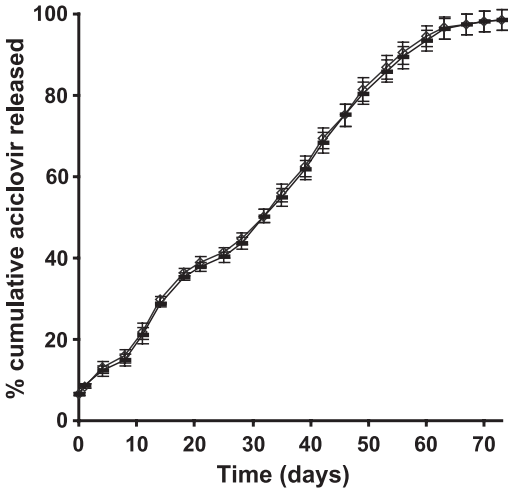

Fig. 6. Release profiles from 10 mg microspheres in 3 ml PBS before (♦) and after (-) exposition to an effective dose (25 kGy) of γ-irradiation.

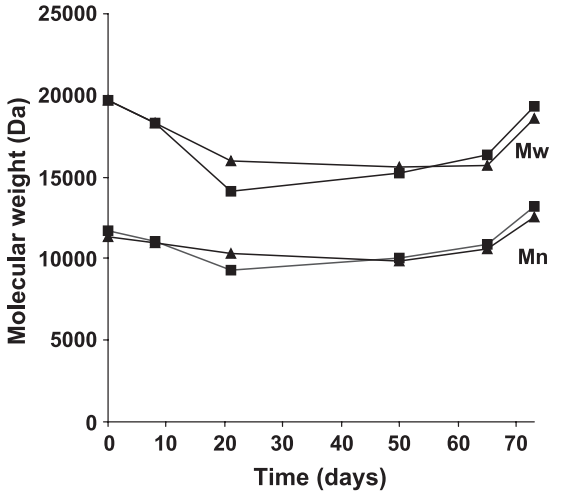

Fig. 7. Evolution of mean molecular weights (*Mw* and *Mn*) of sterilised microparticles (■) and non-sterilised microparticles (▲) upon exposure to phosphate buffer pH 7.4 at 37 °C over a period of 73 days.

equation [27] and there were no significative differences before and after sterilisation ($P<0.05$). The release constant value is an important factor to calculate the amount of aciclovir-loaded microspheres to be administered by the intraocular route. The quantity of microspheres must be reduced as much as possible in order to minimise local toxicity reactions and microspheres must keep their properties after sterilisation.

Irradiation has no statistically significative effect on the percentage of aciclovir released at the end of the assay ($P<0.05$). In conclusion, gamma-sterilisation at a dose of 25 kGy did not affect the release rate of the drug.

Fig. 7 shows the mean *Mw* and *Mn* for sterilised and non-sterilised microspheres. Before sterilisation, *Mw* for polymer and non-loaded microspheres were practically the same, so the solvent evaporation process had no influence on the polymer molecular weight, as observed in DSC studies. Aciclovir-loaded microspheres showed the typical peaks for polymer and aciclovir. GPC was applied to determine changes in molecular weight of samples upon exposure to γ-irradiation.

From the results obtained, the degradation process can be divided into three phases. The first phase (0–20th day) corresponds to polymer decomposition, being evident a decrease in *Mw* and *Mn*. In this phase, *Mw* decreased faster than *Mn*. According to literature [15], this fact supports random chain scission as the mechanism for hydrolytic copolymer degradation, with on the average smaller chains larger than oligomers and monomers remaining. In the following phase, the molecular weight remained quite stable. A possible explanation for this stabilisation is that *Mw* is measured on the solid fraction of the polymer and low molecular weight molecules of the polymer should dissolve in the test liquid and be removed from the solid fraction so they are no more taken into account for *Mw* calculation (approximately until 60th day). During the third phase, which corresponds to last 8 days, an *Mw* increase can be observed because higher amounts of soluble monomers and oligomers are being released.

Table 1
f_2 values for sterilised batches of microspheres

Batch number	f_2 sterilised microspheres
1	97.60
2	94.73
3	96.61
4	99.45
5	99.53
6	99.81
7	92.78

Ionising radiation, such as γ-radiation, produces ionising phenomena, which can lead to a higher molecular reactivity. This process can be favoured by a rise in sample temperature generated by γ-irradiation. As reported, γ-irradiation at room temperature on microspheres made of PLGA of low and high molecular weights [28–30] caused a decrease in *Mw* and *Mn*. Molecular weight decrease was greater in γ-irradiated than non-irradiated microspheres [14]. In the present study, only a slight decrease of *Mw* was observed. As known, the hydrolytic degradation process is accelerated by heat and sample protection with dry ice prevents a temperature increase. In our case, there was no evidence about this fact avoided the initial molecular weight decrease, in particular non-loaded microspheres (PLGA Mw ~ 30,000 Da). We consider drug incorporated into microparticles plays an important role on microsphere behaviour after irradiation. Aciclovir was incorporated into microspheres as a suspension, which improved PLGA stability. On the other hand, PLGA used in this study had a low molecular weight and was probably less susceptible to be altered than high molecular weight polymers.

4. Conclusion

The sterilisation process reported in this work maintained the initial conditions of aciclovir PLGA microspheres. This method allowed final sterilisation of the formulation avoiding the risk of contamination during the microencapsulation process. This effect could be due to three factors: use of a low molecular weight PLGA, protection of samples during the γ-irradiation exposure, and drug incorporation as a suspension. The results obtained in this work, let us conclude that the selected method of sterilisation can be considered excellent because microspheres did not suffer any change due to the γ-irradiation process. The favourable properties of aciclovir microspheres make them suitable systems for the intravitreal treatment of herpes virus infections, in an animal model.

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