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Radiosterilisation of indomethacin PLGA/PEG-derivative microspheres: Protective effects of low temperature during gamma-irradiation

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Abstract

Currently, γ-irradiation seems to be a good method for sterilising drug delivery systems made from biodegradable polymers. The γ-irradiation of microspheres can cause several physicochemical changes in the polymeric matrix. These modifications are affected by the temperature, irradiation dose and nature of the encapsulated drug and additives. This study has aimed to evaluate the influence of temperature during the sterilisation process by gamma irradiation in indomethacin PLGA microspheres including a PEG-derivative. Microspheres were prepared by the solvent evaporation method from o/w emulsion and were then exposed to γ-irradiation. A dose of 25 kGy was used to ensure effective sterilisation. Some microspheres were sterilised with dry ice protection that guaranteed a low temperature during the process whilst others were sterilised without such dry ice protection. The effects of γ-irradiation on the characteristics of non-loaded PLGA/PEG-derivative and indomethacin loaded PLGA/PEG-derivative microspheres with and without protection were studied. Non-protected microspheres showed changes in their morphological surface, polymer glass transition temperature, molecular weight and release rate of indomethacin after sterilisation. However, microspheres sterilised with protection did not show significant differences after γ-irradiation exposure. The sterilisation method was satisfactory when the indomethacin loaded microspheres including a PEG-derivative were exposed to γ-irradiation at low temperature.

Keywords: Microspheres; PLGA; Intraarticular; PEG-derivative; Gamma irradiation

1. Introduction

Current research into the controlled delivery of pharmaceuticals involves the use of biodegradable polymers. The aliphatic polyesters based on lactic and glycolic acids have demonstrated good biocompatibility and the absence of significant toxicity (Menei et al., 1993; Rice et al., 1978) and they have been widely used to prepare injectable delivery systems such as microspheres. Pharmaceutical systems intended for parenteral administration have to meet the pharmacopoeia requirements of sterility. The chemical liability of formulation constituents limits the use of sterilisation methods to obtain an acceptable final sterile product. It is well known that a terminal sterilisation procedure is preferred over aseptic processing, because it is easier from a technological point of view. Sterilisation techniques, such as steam or dry heat cannot be used for biodegradable aliphatic polyesters such as polylactic-co-glycolic acid (PLGA) since they alter the physical and chemical properties of the biomaterial. In its turn, chemical sterilisation with ethylene oxide causes serious toxicological problems, due to residual components of the sterilising agent. Currently, γ-irradiation seems to be a good alternative for final sterilisation of drug delivery systems made from biodegradable polymers. The advantages of gamma irradiation include 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 sterilising pharmaceutical products without providing any biological validation (USP 28, 2005; Montanari et al., 2001).

The effects of γ-irradiation on PLGA polymers and derived formulations have been the subject of several works. For example, it has been observed that gamma irradiation can induce several physicochemical changes in the polymer such as cross-linking, chain scission, formation of radicals and a drop in the polymer molecular weight being the most reported effect (Geze et al., 2001). The decrease in the molecular weight of the PLGA

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is conditioned by the temperature, irradiation dose and nature of the encapsulated drug. Generally, these structural modifications lead to a change in the release rate of the drug and they have been the subject of several studies. In fact, the release rate of clonazepam from PLGA microspheres was increased by approximately 10% after their sterilisation by gamma irradiation using a dose of 25 kGy (Montanari et al., 2001). In this case the process was carried out in air at room temperature. In another report, Yip et al. (2003) observed a drastic modification to the release profile of etanidazole microparticles made with a mixture of PLGA/PLA (PLGA M_w = 40,000; 75,000 Da and PLA M_w = 90,000; 120,000 Da) after γ irradiation (25 kGy) at low temperature (−78 °C). Nevertheless, the same irradiation dose (25 kGy) did not produce changes in low molecular weight PLGA microspheres. In this experiments by Yip et al. a low temperature was maintained during the whole irradiation process, by protecting the samples with dry ice. Nevertheless, under these previous conditions no significant differences were observed between the release profiles of sterilised and non-sterilised PLGA microspheres loaded with ganciclovir (PLGA M_w = 34,000 Da) and aciclovir (PLGA M_w = 12,000 Da) (Herrero-Vanrell et al., 2000; Martinez-Sancho et al., 2004). In both cases, the drug was incorporated in the microspheres as a suspension and the initial properties of the particles were maintained. Similar results were obtained for ibuprofen PLGA microspheres (M_w = 12,000 Da). In this case, the drug was practically dissolved in the polymeric matrix (Fernández-Carballido et al., 2004a), demonstrating that the conditions employed were suitable for low molecular weight PLGA microspheres including drugs which are at different physical states in the polymeric matrix. Taking into account the fact that the same irradiation dose was employed in all cases, the results mentioned above can be attributed to the use of low molecular weight PLGA polymers and low temperature during the process.

The effect of γ-irradiation on the PLGA molecular weight is also influenced by the nature of the drug but it is not easily predicted due to the varying chemico-physical characteristics of the active substance and its interaction with the polymer. In some cases the drop in polymer molecular weight occurred independent of the active molecule included in the formulation. However, in other studies the entrapped drug accelerated polymer degradation usually when the drug is an acidic substance which could act as catalyst agent (Lin et al., 2000; Li et al., 1996; O'Donell and Mcginity, 1998).

Another important point relates to the presence of additives in the formulation. These kinds of agents have been shown to modify the initial characteristics of microspheres such as morphology, drug encapsulation efficiency and in vitro release of the active substance (Hedberg et al., 2002; Lee et al., 2002a,b). Also, alterations in the glass transition temperature (T_g) of the polymers have been observed. Taking into account that a great number of these agents are oils, they may be altered by temperature changes.

Indomethacin (IM, γ-indomethacin; 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid) is an acidic drug with analgesic and anti-inflammatory properties. This non-steroidal agent is sparingly soluble in water and can exist in several crystalline forms but generally, it has been used in pharmaceutical preparations as Form I (type γ). IM has been reported to present physicochemical interactions with several substances (Watanabe et al., 2001).

In this experiment indomethacin biodegradable microspheres have been prepared using a PEG-derivative (Labrafil®) as an additive. The polymer employed has been PLGA 50:50 RG®503 (M_w = 34,000 Da) and oil was used to extend the release time of the drug (Fernández-Carballido et al., 2004b).

Until now, there has been no evidence about the effect of protecting the samples from a rise in temperature, during gamma irradiation exposure in formulations including additives. This study has therefore aimed to evaluate the sterilisation process by gamma irradiation in indomethacin PLGA microspheres including a PEG-derivative. The influence of temperature on the final properties of the formulations was studied. For this purpose, microspheres were exposed to a effective sterilising dose (25 kGy) with and without protection. Samples were protected with dry ice, ensuring a low temperature during the process.

2. Materials and methods

Indomethacin (γ or I form) was supplied by Sigma–Aldrich Chemical (Madrid, Spain). PLGA 50:50 poly(D,L-lactide-co-glycolide) Resomer® RG 503 [M_w = 34,000 Da (GPC)] was purchased from Boehringer Ingelheim (Ingelheim, Germany). PEG- derivative, Labrafil® M 1944 CS was supplied by Cattefossé (Saint-Priest, France). Polyvinyl alcohol (PVA) [M_w = 49,000 Da] was supplied from Sigma–Aldrich Chemical (Madrid, Spain). Dichloromethane and methanol, analytical grade were provided by Merck (Darmstadt, Germany). Distilled and deionized water (Millipore Corporation, Bedford, MA, USA) was used in the preparation of all buffers and solutions.

2.1. Preparation of microspheres

Microspheres were prepared by the solvent evaporation method based on an oil-in-water (o/w) emulsion. The polymer PLGA (200 mg), indomethacin (40 mg) and PEG-derivative (20 μl) were dissolved in 1 ml CH_2Cl_2. The oil was added in the inner phase of emulsion. The external phase of the emulsion was a solution of 1% PVA in distilled water. Once prepared the organic phase was poured into 5 ml of the aqueous phase. The resulting emulsion was stirred using a Polytron homogenizer (Kinematica, Lucerne, Switzerland) at a speed setting of 2000 rpm for 2 min. Upon formation of the emulsion 6 ml of distilled water was added and stirred continuously at the same speed setting for 1 min. Then, the immature microspheres were suspended in 250 ml of distilled water and the system was agitated for 4 h at room temperature, to allow complete evaporation of the organic solvent. Finally, the microspheres were filtered under vacuum using a 5 μm filter and placed in a vacuum desiccator at 25 °C for at least 48 h.