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The effect of γ-irradiation on PLGA/PEG microspheres containing ovalbumin

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Abstract

Poly(ethylene glycol) (PEG) and sodium chloride (NaCl) are excipients used in PLGA microsphere preparation to stabilize proteins and reduce their burst release. No information is till now available in the literature on the effect due to the use of such excipients on the biopharmaceutical performance of γ-irradiated microparticulate systems. On this purpose, different batches of microspheres containing ovalbumin (OVA) were prepared by using a PLGA 50:50 (average Mr: 13000), different amounts of PEG (Mr: 400 or 4000) and/or sodium chloride. The non-irradiated and irradiated microspheres were characterized in terms of morphology (SEM, particle size distribution), OVA and PEG content and in vitro OVA release. Radiolysis mechanisms of OVA and OVA loaded microspheres were investigated by EPR analysis.

Gamma irradiation affects either microsphere morphology or the release of OVA as a function of the amount of PEG, and the use of NaCl. Irradiation significantly reduces release rate of protein from the microspheres containing 15% and 30% of PEG and from controls (microspheres without additives), while no significative effect on protein release rate is highlighted on microspheres containing lower amounts of PEG. EPR investigation shows that increasing amounts of PEG up to 30% have a perturbation effect on OVA radiolysis path.

Keywords: Microspheres; Poly(lactide-co-glycolide); Poly(ethylene glycol); Ovalbumin; Biodegradable polymers; γ-irradiation

1. Introduction

Owing to the development of recombinant DNA technology, a large variety of protein drugs such as hormones, growth factors and vaccines have become commercially available to therapeutic purposes [1]. Proteins are typically administered by injection or infusion because of their poor oral bioavailability. Generally, they have short plasma half-lives and are incapable of diffusing through biological membranes. Many proteins currently being developed are aimed at

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curing chronic conditions where therapy may be required over months or years. Administration by frequent injections to keep the protein drug at effective concentrations is tedious, expensive and has poor patient compliance.

Encapsulation of proteins into microspheres made of biodegradable polymers, e.g. polylactide-co-glicolides (PLGA), offers an opportunity for protein sustained release injections [2]. Such microspheres are mainly produced by double (w/o/w) emulsion solvent evaporation/extraction methods. In these cases, the main problems related to the encapsulation of proteins in PLGA are the formation of aggregates, due to the denaturing conditions rising during microspheres double emulsion preparation processes, and the initial burst release. The literature reports several studies concerning the use of excipients to stabilize proteins and reduce their burst release. Among these excipients PEG and sodium chloride were proposed by several authors [3–9]. PEGs are usually added to the internal aqueous phase to limit the penetration of protein in the interfacial film of the primary w/o emulsion. Consequently, they stabilize the protein by reducing the contact with the organic phase [5]. Sodium chloride, added to the external aqueous phase to generate an osmotic pressure, cause a denser internal structure of the microspheres which reduces the burst release [10].

As the PLGA are thermosensitive materials, the final sterilization of microspheres is performed by means ionising radiations [11]. Ionisation events activate in the microspheres numerous chemical reactions which cause different effect on drug release, depending on the active ingredient used [12–18]. As an example, in the case of insulin-like growth factor-I (rhIGF-I), γ-irradiation causes rhIGF-I aggregation and an increased burst effect in the in vitro drug release experiments [17].

The effects of ionising radiation on PLGA and PLGA microspheres containing different active ingredients as well as the addition of PEGs to PLGA microspheres containing proteins are widely investigated [11–18,4,5,7–9]. However, no information is till now available in the literature on the effect due to the use of such excipients on the biopharmaceutical performance of γ-irradiated microparticulate systems. On this purpose, different batches of microspheres containing ovalbumin (OVA) were prepared by using PLGA 50:50, different amounts of PEGs and/or sodium chloride. The microspheres were γ-irradiated at a dose of 25 kGy [19]. Microsphere characterisation in terms of size, surface morphology, encapsulation efficiency, in vitro release of encapsulated ovalbumin has been performed before and after irradiation. Moreover, the intermediate radicals in the radiolytic process were investigated by matrix EPR spectroscopy after gamma irradiation at 77 K followed by gradual thermal annealing at room temperature. The interpretation of the spectra afforded information on the nature and reactions of the primary species and the interactions of the components in the irradiated microspheres.

2. Materials and methods

2.1. Materials

Poly(lactide-co-glycolide) (PLGA) Medi-sorb®, grade 50:50 DL-2A, inherent viscosity 0.16 dl/g, 13,000 Da Mw, T_g 39.9 °C, was from Alkermes Medisorb® Polymer (Wilmington Ohio, USA).

Sodium chloride (NaCl) was supplied by Carlo Erba (Milan, Italy), polyethylene glycols (PEG) (Mw 400 and 4000 Da), polyvinyl alcohol (PVA) (87–89% hydrolyzed, Mw of 85,000–146,000 Da) and ovalbumin (OVA) (Mw 44,287 Da, p*I* 4.63) were obtained from Sigma-Aldrich (Milan, Italy).

Unless specified, all other compounds were of analytical grade.

2.2. Microsphere preparation

All microspheres were prepared using a double emulsion solvent evaporation method, ovalbumin was chosen as the model protein for these studies because of its good water solubility, its high molecular weight and its thermal stability. Placebo batches were prepared for each composition of protein loaded microspheres (PL_1–PL_6).

An aqueous dispersion (phase W_1) of the protein (55 mg/ml) was dropped into 12 g of the organic solution containing the polymer (10% w/w) dissolved in methylene chloride (phase O).

Emulsification was performed at 9500 rpm with a homogenizer (Ultraturrax model T25 S25NI8G), the system was ice-cooled during the process. This first W_1/O emulsion was dispersed, under mechanical stir-