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Lincomycin hydrochloride loaded albumin microspheres for controlled drug release, produced by Supercritical Assisted Atomization

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ABSTRACT

Supercritical Assisted Atomization (SAA) is proposed for the production of nanostructured microparticles of Bovine Serum Albumin (BSA) loaded with lincomycin hydrochloride (lincoHCl). The process is used to coprecipitate BSA and lincoHCl, producing thermal denaturated BSA microparticles, entrapping the drug in the protein matrix. Several lincoHCl/BSA ratios in water solutions were processed, to produce protein microspheres with different size and drug content. SAA precipitation temperature was set as 100 °C to obtain BSA coagulation and efficient entrapping of lincoHCl. Spherical microparticles showed no coalescence and were produced in all cases studied, with a mean particle size in the range 1–2 μm and loading efficiencies between 87 and 90%. The microspheres produced by SAA showed a controlled release of the drug over about 6 days.

1. Introduction

Lincomycin hydrochloride (lincoHCl) is an antibiotic of the group of licosamides and is used for human and veterinary medical applications. The active principle works against most gram positive bacteria, like *Staphylococcus* sp., *Streptococcus* sp., *Clostridium* sp., *Bacillus anthracis* and *Corynebacterium* sp., which usually can also be treated with penicillin or erythromycin. It is effective for the treatment of infectious diseases, like acne, forunculosis, burns and wounds [1–4]. LincoHCl is mainly used in injection and capsule formulations, although its efficacy in topical applications as gel, paste or aqueous spray has been proved [3,5]. The topical administration has the advantage of delivering the drug directly to the site of action, whereas a prolonged release of the drug can be tailored in order to improve the drug efficacy [6]. Polymeric microparticles that swell in presence of wound fluid, forming in situ hydrogels, can naturally adapt to the shape of the wound, therefore the probability of the formation of compartments, easily attacked by bacteria, is reduced [7–10]. If the polymeric carrier is adequately chosen, the pharmacokinetic profile of the loaded drug can be improved and its efficacy prolonged [11–14].

Among all the available carriers, albumin is an ideal material to produce particles for drug delivery due to its nontoxic, non-immunogenic, biocompatible and biodegradable properties [15]. Additionally, albumin particles show high binding capacity and no serious side effect are evidenced [16–18]. Moreover, the protein shows the properties of a natural polymer: when used as a carrier, allows a controlled and sustained drug release [19,20]. Bovine Serum Albumin (BSA) is able to form complexes and has been extensively used as a matrix for the encapsulation of several drugs [15,16,21–26]. It has a great affinity with hydrophilic compounds and, since it fast solubilizes in water, cannot be used for the controlled release without modifications. Heat treatments of BSA at high temperatures for long time generate intramolecular crosslinks and the new bonds give to the protein properties of hydrolytic resistance [27]. Thermal denaturation concerns the secondary structure of BSA, modifying the content of α-helix and β-sheet. Increasing the temperature, at 50 °C the α-helices have a reversible unfolding and above 65 °C the unfolding induces the formation of irreversible disulphide bridges [28].

Drug/BSA formulations can be prepared using several techniques: emulsion polymerization and stabilization using glu-

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taraldehyde, emulsion formulation with thermal crosslinking, pH-coacervation coupled with glutaraldehyde-ethanol cross-linking and emulsification-dispersion with high pressure homogenization, in which the shear forces induce oxidation and crosslinking [29–32]. Spray drying has been used to produce composite particles of active principles/BSA, obtaining thermal stabilization of the protein during particle drying and using glutaraldehyde as cross-linker [22,24,25]. All these techniques require a second process step for the formation of crosslinking, some of them use organic solvents and need a post processing for solvents and cross-linker removal; furthermore, it is difficult to control the particle size distribution.

To overcome the limitations of traditional techniques, supercritical fluid based processes have been developed for several applications [33–35]. In particular, Supercritical Antisolvent (SAS), Supercritical Assisted Atomization (SAA) and Supercritical Extraction from Emulsion (SEE) have been developed to produce micro and nanoparticles [36–39]. SAA has been successfully used for the production of coprecipitates, as nanosctructured microparticles formed by a carrier in which the active principle is uniformly distributed [40–43] or as polymeric microparticles loaded with nanoparticles [44,45].

In SAA process, supercritical carbon dioxide (SC-CO_2) is intimately mixed with the liquid solution containing the compound to be micronized in a high pressure vessel, in which the conditions of temperature and pressure are selected to get an expanded liquid solution. This solution, characterized by reduced viscosity and surface tension, is sprayed in a precipitator, obtaining an effective atomization. The droplets are dried using nitrogen at adequate temperature, that continuously flows in the precipitator during the injection of the solution [46–49]. SAA equipped with a vacuum system has been previously applied to produce BSA microparticles using precipitation temperatures of 70 °C and 60 °C. At the used conditions, no modification or denaturation of the protein was detected [50,51]. Using higher precipitation temperatures, it is possible to denaturate the BSA, with the consequent possibility to use it as a carrier for controlled and delayed release of hydrophilic drugs, as in the case of gentamicin/BSA [40]. SAA technique defeats the difficulties of the traditional techniques in controlling particle size and distribution of the coprecipitate and induces, during atomization, the denaturation of BSA with no use of chemical crosslinkers and post processing.

In this work, we want to obtain nanostructured microparticles of BSA loaded with lincoHCl for the controlled release of this drug for topical applications, using SAA technique. Optimizing the operating conditions of the process, it is possible to have the stabilization of BSA due to the decrease of pH in the mixer during the solubilisation of SC-CO_2 in the aqueous solution and by the thermal effect during precipitation and particle drying [52]. Particle size can be in the appropriate range for transdermal controlled release formulations, that can be obtained by direct spray or spreading the microparticulate powder directly on the wounds or incorporating the particles into gels, foams or pastes. Therefore, we will try to produce the composite microparticles in the range 1-5 μm, suitable for transdermal controlled release to be used for direct spraying on the wounds.

2. Materials and methods

Bovine Serum Albumin (BSA) and lincomycin hydrochloride (lincoHCl) were supplied by Sigma-Aldrich (Milan, Italy). Water (HPLC grade) with a purity of 99.5% was supplied by Sigma-Aldrich (Milan, Italy). Carbon dioxide (CO_2; purity 99.9%) and Nitrogen (N_2; purity 99.9%) were purchased from SON (Naples, Italy).

SAA laboratory apparatus used is composed by two high-pressure pumps (mod. 305, Gilson, Villiers Le Bel France) to deliver the water solution and the liquid CO_2 to the saturator. The saturator is a heated high pressure packed vessel (volume: 25 cm^3) which assures a large contact surface between liquid solution and CO_2. The expanded liquid obtained in the saturator is sprayed through a nozzle into the precipitator (volume: 3 dm^3) that operates at atmospheric pressure. A controlled heated flow of N_2 (about 1200 nL/h) is flown to the precipitator to enhance the evaporation of water from the droplets. A sintered filter at the bottom of the precipitator, with a porosity of 0.5 μm, allows the collection of the powder and the flowing through of the gases. SAA apparatus layout and further details can be found in previous papers [51,53].

The morphology of BSA and lincoHCl-BSA loaded particles has been analysed by a Field Emission Scanning Electron Microscope (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). Powders were dispersed on a carbon tab previously stuck to an aluminium stub (Agar Scientific, United Kingdom). Samples were coated with gold (layer thickness 250 A) using a sputter coater (mod. 108 Å, Agar Scientific, Stansted, United Kingdom). At least 20 SEM images were taken for each batch to verify the powder uniformity.

Particle size (PS) and particle size distribution (PSD) were measured from FESEM images using the Sigma Scan Pro Software (rel. 5.0, Jandel Scientific, Erkrath, Germany). Histograms representing PSDs in terms of particles number and volumetric cumulative were best fitted using Microcal Origin Software (rel. 8.0, Microcal Software, Inc., Northampton, MA).

SAA coprecipitates were also characterized by microanalysis to investigate their chemical structure. Elemental analysis and element mapping were performed using the FESEM equipped with an energy dispersive X-ray spectroscopy (EDX, INCA Energy 350, Oxford Instruments, Witney, United Kingdom).

Diffraction patterns of co-precipitated powders were obtained using an X-ray diffractometer (mod. D8 Discover, Bruker AXS, Inc., Madison, USA). The measuring conditions were as follows: Ni-filtered CuK radiation, λ = 1.54 Å, 2θ angle ranging between 5° and 60° with a scan rate of 3 s/step and a step size of 0.2°.

Thermograms of powder samples were obtained using a differential scanning calorimeter (DSC mod. TC11, Mettler Toledo, Inc., Columbus, USA). The samples (about 4 mg) were heated from 25 to 200 °C at 5 °C/min, under a nitrogen purge of 50 mL/min.

Fourier Transform Infrared (FT-IR) spectra were obtained via M2000 FTIR (MIDAC Co), at a resolution of 0.5 cm^{-1}. The scan wavenumber range was 4000–400 cm^{-1}, and 16 scan signals were averaged to reduce the noise. Powder samples were ground and mixed thoroughly with potassium bromide (KBr) as infrared transparent matrix.

Drug loading was studied using Pharmacopoeia HPLC (High Performance Liquid Chromatography) method (USP 29) [54] with some modifications for the measurement of the lincoHCl drug content. Samples of lincoHCl/BSA powder were dissolved under vigorous stirring in a solution water-acetic acid 1% v/v (pH 4) at 37 °C. The solution was sonicated for 30 min and stored for 24 h to obtain the complete release of the drug from the carrier. The obtained solution was filtered to eliminate the BSA residue and diluted with water to increase the pH and analysed by HPLC-UV/vis (Hewlett-Packard model G131-132, USA). The column used is a reverse phase C18 column (4.6 mm × 250 mm; 5 mm particle size; 80 Å pore size; Hypersil BDS RP-C18); it was equilibrated at a flow rate of 1 mL/min with a mobile phase consisting of phosphate buffer pH 7.0 and acetonitrile (ratio 75:25 v/v). LincoHCl was monitored at 204 nm with a retention time of 10 min. Loading efficiency was calculated as the ratio of the drug content in the produced particles over the drug loaded at the beginning of the process.

The drug release rate was performed using a 0.1 M Phosphate Buffer Solution (PBS) at pH 7.0 as dissolution medium. 100 mg of powder was suspended in 2 mL of PBS with 0.5% w/w of tween 80

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