Supercritical assisted atomization gentamicin microparticles for topical drug controlled release

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INTRODUCTION

A wound infection is defined as a homeostatic imbalance between the host tissue and the presence of microorganisms at a concentration that exceeds 10^5 organisms per gram of tissue (Sussman & Bates-Jensen, 2001). An infection can be associated with a large variety of occurrences, from traumatic event and burns to chronic ulcers and complications derived by surgery or device implantations (Baranoski & Ayello, 2008). The main goal of treating the various types of wound infections is to reduce the bacterial charge in the wound in order to ease the healing processes of the organism.

Antibiotics delivered by oral route suffer from the drawbacks of general systemic toxicity and of poor penetration into ischemic and necrotic tissue typical of posttraumatic events (Campton Johnston & Wilson, 2001)

Topical delivery of antibiotics by topical administration can solve most of the disadvantages of systemic administration, maintaining an high local concentration for prolonged time, without exceeding systemic toxicity (Stigter et al, 2004).

Different biodegradable carriers from both natural and synthetic polymers have been proposed as delivery systems for antibiotics carriers. Among the natural polymers, dextran such as alginate and pectin are largely used in preparation of microspheres for systemic as well as locally drug sustained release. In fact, they have received wide attention, as ideal drug carriers, because of their biocompatibility, biodegradability and the ability of some dextrans to enhance the healing process by inducing cytokine production by human monocytes, resulting in the increase of the autogenic wound healing. Microparticles loaded with antibiotics have been produced by different micro-encapsulation techniques in order to achieve tailored drug release.

Supercritical assisted atomization (SAA) technology has been proposed as an alternative to conventional spray drying technology for the production of microparticles and microspheres, particularly, using supercritical carbon dioxide ($SC\text{-}CO_2$). The SAA process has already been successfully tested for the micronization of some pharmaceutical compounds obtaining particle in the range of 1–3 μm with narrow size distribution (Della Porta et al, 2010). Recently, the SAA technique has been also applied to the production of biopolymer based microparticles for controlled drug delivery.

In this study, the SAA technique is proposed for the production of dextrans based microspheres loaded with gentamicin sulfate (GS) for topical application, in order to obtain a sustained release of the drug. GS has been selected as model antibiotic because of its action against both gram-positive and gram-negative bacteria and could be very useful in the cure of bacterial induced infections. Moreover, the carrier diameter, in the range of 1–3μm, may be useful for charging the formulation in specific fibers, or foams, or gels for wound dressing. Morphological, size distribution, solid state and drug release analyses have been conducted on dextrans/GS microparticles with the aim to investigate the processability by SAA of the drug-polymer blend and to establish the distribution of the drug inside the produced particles. Moreover, the effect of the polymer/drug ratio on drug release profiles is evaluated.

MATERIALS AND METHODS

Alginate, pectin, and GS have been supplied by Sigma–Aldrich (Milan, Italy). Other solvents used are of analytical grade. Carbon dioxide (CO2, purity 99.9%) and nitrogen (N2, purity 99.9%) have been purchased from SON (Naples, Italy).

Supercritical Apparatus

The SAA laboratory apparatus consists of two highpressure pumps delivering the liquid solution and liquid CO_2 to a heated bath and, then, to the saturator. The solution obtained in the saturator is sprayed through a thin wall (80 mm internal diameter) injection nozzle into the precipitator operating at atmospheric pressure. A controlled flow of N2 is taken from a cylinder, heated in an electric heat exchanger and sent to the precipitator to facilitate liquid droplet evaporation. The saturator and the precipitator are electrically heated using thin band heaters. A stainless steel filter located at the bottom of the precipitator allows powder collection and the gaseous stream flow out.

Morphology and Particle Size Distribution

The morphologies of the microparticles were observed by a field emission-scanning electron microscope (FESEM, mod. LEO 1525, Carl Zeiss, Germany). At least 20 SEM images were taken for each run to verify the powder uniformity. Particle size (PS) and the particle size distribution (PSD) were evaluated by light scattering (LS) (Coulter LS 13320, Beckman Coulter, Inc., USA). Analyses were performed on microspheres suspensions using 30mg of each sample dispersed in dichloromethane by sample sonication.

Drug Content and Encapsulation Efficiency

Samples of each manufactured batch were dissolved under vigorous stirring in PBS buffer at 37°C. GS drug content was obtained using Pharmacopoeia HPLC method (USP 30). Briefly, 25mg of GS/Alginate/Pectin powder was stirred in 25mL of PBS buffer (0.1 M) until the powder was completely dissolved. Ten milliliter of the solution were transferred in a suitable test tube, then, 5mL of isopropyl alcohol and 4mL of a previously prepared phthalaldehyde solution were added. The solution was stirred and isopropyl alcohol was added to obtain a 25mL solution. The solution was heated for 15 min in a water bath at 60°C, then cooled at room temperature and analyzed by HPLC at 330 nm (Chromatopac L-10AD system equipped with a Model SPD-10AV UV–vis detector and a Rheodyne Model 7725 injector loop 20 mL, Shimadzu, Tokyo, Japan).

Drug Release Studies

Drug release experiments were performed in vertical Franz-type diffusion cells (Microglass, Naples, Italy), with an exposed surface area of 0.6 cm^2. A cellulose acetate filter with pore size 0.45 mm (Sartorius, Goettingen, Germany) was used as the barrier. The donor compartment was filled with 25mg of GS/alginate/pectin formulation and 100 mL of the receptor phase. The receptor phase was 0.1M PBS buffer solution, thermostated at 37°C and magnetically stirred in order to prevent any boundary layer effects. The receptor solution was sampled, derivatized and analyzed by HPLC (USP 30, gentamicin content method) for the determination of permeated GS. Moreover, microbiological activity of gentamicin formulations was evaluated in vitro against gram positive bacteria.

RESULTS AND DISCUSSION

Microparticles loaded with GS were manufactured using gentamicin-alginate-pectin ratio between 1:1:1 and 1:3:1, using 1.0% (w/w) solution concentration as feed solutions. All manufactured microspheres were in a narrow size distribution with a mean diameter between 1.5 and 2.0 μm. Diameter, as well as surface roughness of the microparticles were dependent on drug/polymers ratio. Particularly, the higher was the ratio the smaller was the mean diameter and smoother was the particles surface.

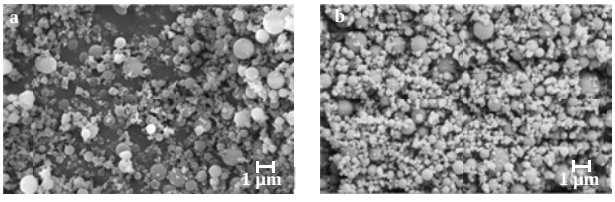

Figure 1: SEM images of GS/alginate/pectin microparticles obtained by SAA from water solutions at different GS/polymer ratio: (a) 1:1:1, (b) 1:3:1.

All microparticles batches exhibited good content and very high encapsulation efficiency (about 100%). Moreover, encapsulation process prevented water uptake by gentamicin that in normal conditions is very hygroscopic and able to attract moisture from air till to melt.

Drug release profiles showed a prolonged release of GS leading to a complete release of the drug between 3 and 6 days depending on the drug/polymers ratio. In fact, a higher amount of polymer materials in the microspheres produced a slower release of the drug, since alginate and pectin blend acts as a barrier to the fast diffusion of GS. Moreover, SAA microparticles showed an initial burst effect (till 6 hours) suitable to prevent infection spreading very important at the beginning of an antibiotic therapy.

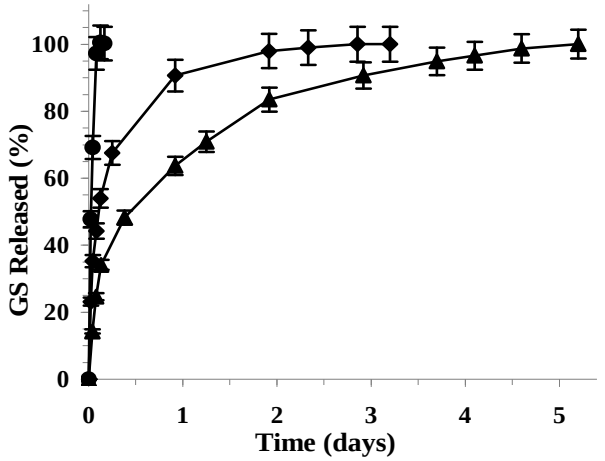

Figure 2: Release profiles of GS/alginate/pectin microparticles manufactured by SAA with different GS/polymer ratio: (-▲-) 1:3:1, (-◆-) 1:1:1, (-●-) pure GS.

Preliminary microbiological studies conducted in vitro against different gram positive bacteria indicate that the activity of gentamicin is stronger for SAA formulations with higher polymer blend concentration that are able to guarantee an appropriate burst effect and a prolonged release of the drug able to maintain good antibiotic concentration over time, in accordance to permeation release profiles.

CONCLUSIONS

SAA technique was able to produce microparticulate carriers based on dextrans for the controlled release of gentamicin sulfate. Microparticles were obtained with mean diameter ranging between 1-3 μm with narrow size distribution. Mean diameters was correlated with polymer blend concentration used to manufacture the microparticles. In fact, the higher was the polymer concentration the smaller was the particle diameters.

Permeation profiles demonstrated that SAA microparticles were able to prolong gentamicin antibiotic effect compared to pure drug. Moreover, drug permeation profiles resulted dependent on GS/alginate/pectin ratio used to prepare feed solutions. In fact, microparticles produced with 1:3:1 ratio were able to prolong the release rate of the drug till 5 days while 1:1:1 microparticles achieved total release of the gentamicin in 3 days. Thus, formulations with GS/alginate/pectin ratio of 1:3:1 would be very useful for treatment of wound infections because they could be able to maintain the antibiotic concentration over the minimal inhibition concentration for various pathogens for more than a week of treatment.

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