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Preparation of chitosan microparticles with diverse molecular weights using supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer

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ABSTRACT

Supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer (SAA-HCM) was used to prepare chitosan microparticles with the well-defined spherical morphology and the controlled particle size distribution as a promising carrier for drug delivery system. Chitosan with different molecular weights (3 kDa, 50 kDa, 300 kDa) was successfully micronized, in which water was used as the solvent for 3 kDa chitosan, while 1.0% (v/v) acetic acid aqueous solution was used for 50 kDa and 300 kDa chitosan, respectively. The process parameters including operating pressure and temperature in the mixer, mass flow ratio of CO_2/solution, precipitator temperature and solution concentration were explored to evaluate their influences on the morphologies and size distributions of precipitated particles. Results showed that particle size tailoring (ranging between 0.2 and 5 μm) could be achieved through modulation of the process parameters. After processing by SAA-HCM, Fourier transform infrared spectroscopy did not indicate significant change in the main structure of chitosan microparticles. Compared with raw materials, a decrease in crystallinity and thermal stability was observed for the SAA-HCM processed chitosan microparticles, as demonstrated by X-ray diffraction, thermogravimetric analysis and differential scanning calorimetry.

1. Introduction

Micro-/nano-particulate drug delivery system possesses several advantages over the conventional dosage forms, such as higher efficacy, reduced fluctuation in circulating drug levels, and improved patient compliance [1,2]. These particles can be administered via parenteral, nasal or pulmonary routes in the forms of powders, suspensions or aerosols. Through the proper complex of biodegradable and biocompatible polymeric materials with drugs, controlled release of the therapeutic agent is achievable in microsphere-based therapies. Among the potential polymers, chitosan is of particular attraction as carriers for drug delivery system.

Chitosan is a natural linear polysaccharide derived by deacetylation of chitin which is the second most abundant biopolymer found in nature next to cellulose [3]. Owing to its favorable biological properties, such as hydrophilicity, biodegradability, good biocompatibility and low toxicity, chitosan has drawn increasing attentions in recent years and been widely used in many fields, especially in biomedical engineering and pharmaceutical formulations [4,5]. Chitosan derivatives as well as chitosan have been extensively studied for targeted delivery of therapeutic antigens and proteins particularly via mucosal routes due to their excellent mucoadhesive and absorption-enhancing properties [6]. On the one hand, chitosan can interact with mucus and epithelial cells, and finally result in opening of cellular tight junctions thus increasing the paracellular permeability of the epithelia. On the other hand, other structural elements of this polymer likely contribute to their penetration-enhancing activity [7].

Despite many merits of microparticles, the control of particle size is quite essential for production of particles suitable for targeted drug delivery. For instance, microparticles with aerodynamic diameters of 1–5 μm can be delivered into deep lungs without earlier deposition or exhalation, which is called aerosol delivery formulation [8]. In the pharmaceutical industry, conventional processes e.g. milling, spray-drying, ionic gelation, solvent evaporation, and reverse micelles formation, have been used for production of microparticles [2,9]. However, these traditional methods somehow have several drawbacks, such as high disposal temperature, use of organic solvents, and poor control of particle size and particle size distribution (PSD). In the past few decades, several supercritical fluid (SCF)-based techniques were proposed to prepare microparticles to overcome the limits of traditional micronization methods. Among the SCF-based techniques, supercritical fluid assisted atomization (SAA) [10] is highlighted with its availability for not only organic solvent/drug systems, but also aqueous solutions of water-soluble drugs. SAA process used a saturator to achieve the mixing of SC–CO_2 and liquid solution, a thin wall injector to induce the atomization

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of the solution and a precipitator to obtain the dried particles. This process has been used for micronization of inorganic salts [11], antibiotics [12], polymers [13], proteins [14] and drug loaded composites [15] from either organic solvents or aqueous solutions with controlled PSDs.

Supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer (SAA-HCM) in which an orifice plate is designed as the cavitation generator to intensify the mixing between SC–CO_2 and liquid feedstock, has been developed as an improved SAA process since 2008 [16]. Hydrodynamic cavitation would facilitate the mass transfer in heterogeneous systems, as a result of which, near thermodynamic equilibrium can be reached within a shorter time. Meanwhile, the sufficient mixing of the mixture in the hydrodynamic cavitation mixer can reduce the viscosity and the surface tension of the aqueous solution to some extent, and thus increases the homogeneity of the primary droplets and leads to particles with better control over morphology and particle size [17]. SAA-HCM has also been applied successfully in the micronization of levofloxacin hydrochloride [16], sodium cellulose sulfate [18], Ginkgo flavonoids [19], bovine serum albumin [20] and lysozyme [21] with controlled PSDs.

Micronization of chitosan using SCF-based technologies has been attempted by limited researches. Reverchon et al. micronized chitosan from 1.0% acetic acid aqueous solution using SAA process [22], and furthermore, non-coalescing spherical ampicillin trihydrate/chitosan composite microparticles [23] and Fe_3O_4 loaded chitosan microparticles [24] were also obtained. Yet, only a certain molecular weight chitosan was used, and chitosan with different molecular weights has not been taken into account in these studies. Moreover, detailed structural analyses are needed. Commercial chitosan is provided with a wide range of molecular weights. Chitosan with relatively high molecular weight was often used as drug carrier for sustained release in the drug formulation. Some reports showed that chitosan with lower molecular weights had improved water-solubility and some special biological functions, and performed better as an absorption enhancer in the drug delivery [25,26]. To date, SAA-HCM process has not been used to produce chitosan polysaccharide-based microparticles. In this work, the processability of chitosan with different molecular weights using SAA-HCM is verified. For chitosan with low molecular weight, water is chosen as the solvent since it is soluble in aqueous solution. Apart from the molecular weight, process parameters including mixer pressure, mixer temperature, mass flow ratio of CO_2/solution, precipitator temperature and solution concentration which may affect the morphology, particles size and their distribution will be investigated. Also, the influences of SAA-HCM treatment upon the structure, crystalline state and thermal property of the material will be discussed in detail.

2. Experimental

2.1. Materials

Chitosan with different molecular weights (Mw = 3 kDa, 50 kDa, 300 kDa) was purchased from Zhejiang Golden-Shell Pharmaceutical Co. Ltd. (Taizhou, China). The degrees of deacetylation of all the chitosan molecules were about 90%. Among these three kinds of chitosan, the lowest molecular weight chitosan (Mw = 3 kDa) could be dissolved in water properly. Meanwhile, 1.0% (v/v) acetic acid aqueous solution was used to solubilize the other two types of chitosan. Acetic acid (analytical purity) was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Food grade carbon dioxide (CO_2) and nitrogen (N_2) with the purity above 99.0% were supplied by Jingong Gas Co. Ltd. (Hangzhou, China). All reagents were used without any further purification.

2.2. SAA-HCM apparatus

As shown in Fig. 1, the SAA-HCM laboratory apparatus consists of three feed lines for SC–CO_2, liquid solution and hot N_2, respectively, as well as three vessels: mixer, precipitator, and condenser. The operating procedure is described in detail as follows [16].

In the beginning, CO_2 stored in a cylinder was released into the system at gas state through a pressure reducing valve. Before entering into the cooling bath (DFY-20 L/30, Yuhua) to ensure a liquid flow, the CO_2 firstly passed through a filter to remove any impurities. Liquid CO_2 was pressurized by a high pressure pump (P-200A, Thar), and heated in a heating bath (DF-101S, Changcheng) to a desired temperature. The air oven acted as prestorage to ensure a constant feed pressure. Meanwhile, the prepared liquid solution in a glass vessel was pumped by a high pressure pump (J-W, ZJSH) with its feeding temperature controlled by a heat exchanger. The SC–CO_2 and the liquid solution were mixed in the hydrodynamic cavitation mixer whose temperature and pressure were also maintained at stable values. The mixture was then sprayed into the precipitator through a thin wall stainless steel nozzle (i.d. 200 μm) to generate atomization at near atmospheric pressure. N_2 was taken from a cylinder, heated by an electric heat exchanger (NQD, ZJ-Dros) and then sent into the precipitator with a controlled flow rate to facilitate the evaporation. The precipitator was electrically heated to control the temperature. The dried particles were collected by a stainless steel filter with 0.5 μm pore size at the bottom of the precipitator. At the same time the mixture of CO_2, N_2 and solvent vapor passed through the filter, and then reached a cooling unit where the solvent was condensed.

2.3. Analytical methods

2.3.1. Particles morphology and size

Samples of the particles produced under different conditions were observed by a field-emission scanning electron microscope (SEM, Sirion, Netherlands), and SEM images were conducted with a particle image analysis software (Nano Measurer, Fudan University, China) for particle size distribution (PSD) calculation. At least 1500 target particles were considered in each PSD calculation. Experiment under each condition was performed at least twice. PSDs were analyzed using Origin software (release 8.6, OriginLab Corp., USA) and then converted into number distributions by Systat Software (TableCurve 2D 5.01, Systat Software Inc., USA).

2.3.2. Fourier transform infrared spectroscopy (FT-IR)

A Fourier transform infrared spectrometer (FT-IR, Nicolet 5700, USA) was used to characterize the untreated chitosan and chitosan microparticles prepared by SAA-HCM process. Chitosan samples were ground with anhydrous KBr powder and then compressed into a film with an evacuable die. FT-IR spectra of these films were recorded over the wavenumber range of 4000–400 cm^{-1} at ambient temperature with a resolution of 4 cm^{-1}.

2.3.3. X-ray powder diffraction (XRD)

Chitosan samples were analyzed with an XRD apparatus (X'Pert PRO, PANalytical, Netherlands). The generator voltage was 40 kV and the tube current was 40 mA. Cu was used for anode material. 2θ angle varied from 5° to 80° with a scan rate of 10 s/step and a step size of 0.0167°.

2.3.4. Thermogravimetric analysis (TGA)

TGA analyses were performed with a thermogravimetric analyzer (Pyris 1 TGA, Perkin Elmer, USA). Samples of untreated chitosan and chitosan microparticles were loaded on an open platinum TGA pan suspended from a microbalance and heated from 40 to 700 °C with a rate of 10 °C/min under nitrogen atmosphere.

2.3.5. Differential scanning calorimetry (DSC)

DSC analyses were conducted using a differential scanning calorimeter (Pyris DSC 7, Perkin Elmer, USA). Samples of untreated chitosan and processed chitosan microparticles were sealed in aluminum