Carbohydrate Polymers 102 (2014) 400–408

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Supercritical fluid assisted production of chitosan oligomers micrometric powders

Zhe Du, Yu-Bin Shen, Chuan Tang, Yi-Xin Guan*, Shan-Jing Yao, Zi-Qiang Zhu

Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form
19 November 2013
Accepted 21 November 2013
Available online 1 December 2013

Keywords:
Supercritical fluid assisted atomization
Hydrodynamic cavitation mixer
Chitosan oligomers
Micronization

ABSTRACT

Chitosan oligomers (O-chitosan) micrometric particles were produced from aqueous solution using a novel process, i.e. supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer (SAA-HCM). Hydrodynamic cavitation was introduced to enhance mass transfer and facilitate the mixing between SC-CO_2 and liquid solution for fine particles formation. Well defined, separated and spherical microparticles were obtained, and the particles size could be well controlled with narrow distribution ranging from 0.5 μm to 3 μm. XRD patterns showed amorphous structure of O-chitosan microparticles. FTIR, TGA and DSC analyses confirmed that no change in molecular structure and thermal stability after SAA-HCM processing, while the water content was between 5.8% and 8.4%. Finally, tap densities were determined to be below 0.45 g/cm^3 indicating hollow or porous structures of microparticles. By tuning process parameters, theoretical mass median aerodynamic sizes lied inside respirable range of 1–2 μm, which presented the potential of the O-chitosan microparticles in application as inhaled dry powders. SAA-HCM was demonstrated to be very useful in particle size engineering.

1. Introduction

Powder systems composed of microparticles are of great interest in drug formulations where they serve directly as delivery systems or as building blocks of innovative drug products. Microparticulate dosage offers several advantages over conventional drug delivery methods, such as high efficacy and extended administration flexibility (Reverchon & Antonacci, 2006). Depending on the application, powder products should be tailor-made with respect to size distribution and morphology (Buttini, Colombo, Rossi, Sonvico, & Colombo, 2012). For example, the aerodynamic size of powders to be used for dry inhalation therapies should range between 1 and 5 μm (Amidi, Mastrobattista, Jiskoot, & Hennink, 2010). Supercritical fluid (SCF) based micronization processes had been developed with characteristics like mild operation temperature and minimum organic solvent residues and were successfully applied to polymer processing (Knez, Markočič, Novak, & Hrnčič, 2011). Unfortunately, there are still some difficulties in producing microparticles of hydrophilic biopolymers like chitosan or its oligomers (O-chitosan) using SCF based techniques including RESS, PGSS and SAS. On the one hand, these biopolymers have nearly no solubility in SC-CO_2; and on the other hand, SC-CO_2 has hardly any antisolvent effect on their aqueous solutions.

Supercritical fluid assisted atomization (SAA) was proposed by Reverchon (2002), which makes use of the atomization assisting effect of SC-CO_2 to obtain fine particles. This technique has been investigated for micronization of various kinds of polymers, either water soluble or insoluble (Reverchon & Antonacci, 2006, 2007b). The original SAA process has been then improved adding the possibility to work in the precipitator at reduced pressures to work with highly thermolabile compounds (Adami, Liparoti, Izzo, Pappalardo, & Reverchon, 2012; Adami, Liparoti, & Reverchon, 2011; Liparoti, Adami, & Reverchon, 2012). In 2008, our group (Cai, Guan, Yao, & Zhu, 2008) established an improved SAA process and introduced the hydrodynamic cavitation phenomenon to intensify the mixing between SC-CO_2 and solution (SAA-HCM). In SAA-HCM process, an orifice plate is used as the mixing point and the hydrodynamic cavitation generator. The emerging and collapse of CO_2 transient cavities results in convective and enhanced mixing, and a "homogeneous dispersion" is finally formed in the mixer with moderate residence time, which is preferable for preventing polymer from thermal degradation (Wang, Guan, Yao, & Zhu, 2010). So far SAA-HCM process has been successfully employed in micronization of diverse substances with good morphology and size control (Du, Guan, Yao, & Zhu, 2011; Du, Tang, Guan, Yao, & Zhu, 2013; Miao, Yu, Du, Guan, Yao, & Zhu, 2010; Wang, Guan, Yao, & Zhu, 2011). The capability of SAA-HCM to process aqueous solution makes this process promising for chitosan and O-chitosan micronization. Meanwhile, the expansion of CO_2 in the precipitator would reduce water vapor pressure and the inert atmosphere could also prevent oligosaccharides from being oxidized.

* Corresponding author. Tel.: +86 571 87951982; fax: +86 571 87951982.
E-mail address: guanyx@zju.edu.cn (Y.-X. Guan).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.11.029

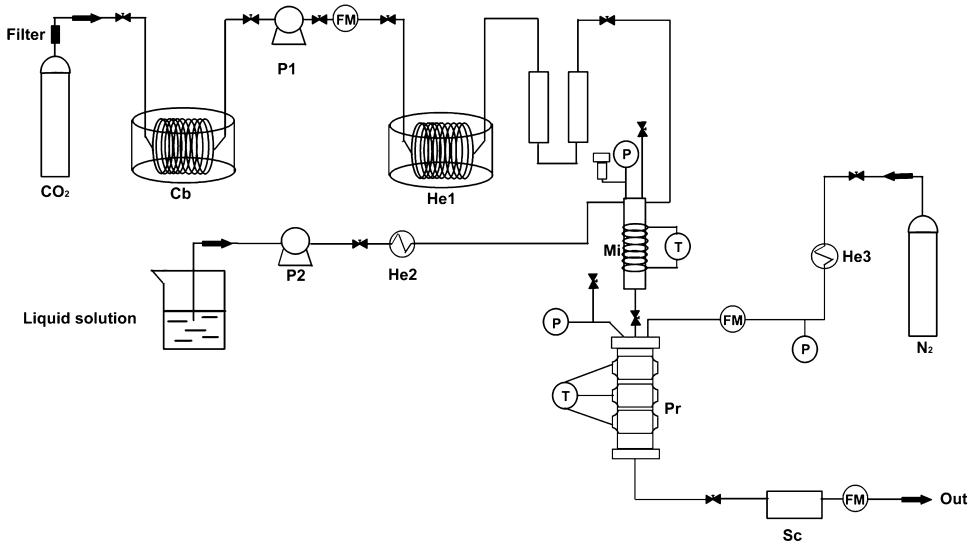

Fig. 1. Schematic diagram of the supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer (SAA-HCM) process (Cb, cooling bath; He1, He2 and He3, heat exchanger; P1 and P2, high pressure pumps; FM, mass flow meter; Mi, mixer; Pr, precipitator; Sc, solvent condenser).

Till now, several studies have been successfully carried out using SAA process to produce chitosan microparticles (Adami & Reverchon, 2012; Reverchon & Adami, 2013; Reverchon & Antonacci, 2006, 2007a). However, these studies all used acidic solutions for chitosan micronization and the concentration range was limited due to the viscosity increase of polymer. Moreover, no O-chitosan was processed. It is well known that O-chitosan has higher water solubility and lower viscosity than chitosan itself, accompanied by enhanced hygroscopicity and susceptibility to processing conditions (Muzzarelli, 2010; Muzzarelli, Stanic, & Ramos, 1999). O-chitosan supplied to wounded human tissues exerts key biochemical effects leading to healing (Muzzarelli, 2009). The aim of this study is to verify the feasibility of SAA-HCM process for O-chitosan micronization using water as solvent. The effects of processing conditions on product properties were elucidated and the influences of the treatment on polymer structure integrity were analyzed to investigate the ability of this process in O-chitosan particle engineering without damaging the molecular structure. The density and theoretical aerodynamic properties was also evaluated to further demonstrate the potential of O-chitosan microparticles in pharmaceutical application.

2. Materials and methods

2.1. Materials

Chitosan oligomers (O-chitosan) with a degree of deacetylation of 91.3% and mass mean molecular weight of 1000 Da were purchased from Qingdao Yunzhou Biochemistry Co. Ltd. (Qingdao, China), and the main components are chitosan pentamer and hexamer. Carbon dioxide (CO_2, purity of 99%) and nitrogen (N_2, purity of 99%) was supplied by Hangzhou Jingong Gas Co. Ltd. (Hangzhou, China). Deionized water was prepared in our laboratory. All other chemicals used were of analytical grade.

2.2. Experimental apparatus

As illustrated in Fig. 1, the SAA-HCM apparatus is characterized by three feed lines, i.e. supercritical CO_2, the solution and the drying gas N_2, respectively, as well as three vessels: the mixer, the precipitator, and the condenser. Liquid CO_2 from a high pressure pump (P1) was heated in a heating bath (He1) to achieve the desired temperature and then sent into the mixer (Mi). Liquid solution was fed by a high pressure pump (P2), after heated (He2) and also delivered into the mixer where SC-CO_2 contacted with the solution. The mixture was sprayed through a thin-wall stainless steel nozzle (i.d. 200 μm) located in the precipitator (Pr) to generate atomization. The precipitator was operated at 0.13 MPa, and a controlled flow of 20 N dm^3/min of N_2 heated by a heat exchanger (He3) was sent into the precipitator to assist the evaporation of solvent. The dried particles were collected by a stainless steel filter with a 0.5 μm pore size at the bottom of the precipitator. The mixture of CO_2, N_2 and solvent vapor then passed through a cooling unit (Sc) to condense the solvent. Detailed description of the layout and operating procedures of this process is presented elsewhere (Cai, Guan, Yao, & Zhu, 2008).

2.3. Particles size and distribution

Samples of O-chitosan microparticles produced were observed by scanning electron microscope (SEM) (Sirion, FEI, Netherlands) to analyze their morphology. For particle size distribution (PSD) calculation, SEM images were conducted with particle image analysis software (Nano Measurer 1.2.5, Fudan University, China). At least 2000 target particles were considered in each PSD calculation performed. The statistical results were fitted by Systat Software (TableCurve 2D 5.01, Systat Software Inc., USA) to give size distribution curves.

2.4. Particles solid state characterizations

A Fourier transform infrared spectrometer (Nicolet 5700, ThermoNicolet, USA) was used to analyze structural stability of O-chitosan after SAA-HCM processing. O-chitosan samples were mixed with anhydrous KBr and compressed into a film with an evacuable die. The films were placed into the nitrogen-purged sample chamber and spectra were recorded. 256 scans between 400 and 4000 cm^{-1} with resolution of 4 cm^{-1} were co-added.

Thermal behavior of O-chitosan samples was analyzed by differential scanning calorimeter (Q200, TA Instruments, USA) and thermogravimetric analyzer (Pyris 1, Perkin Elmer, USA), respectively. For DSC, samples of unprocessed O-chitosan and