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Preparation of ultrafine chitosan particles by reverse microemulsion

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Abstract

Ultrafine chitosan particles were prepared by reverse microemulsion consisting of water, Triton X-100, octanol and cyclohexane. Two methods of preparing ultrafine chitosan particles were adopted and compared using TEM and IR, and possible mechanisms for the formation of ultrafine chitosan particles were proposed. Experimental results show that the method which combined ionic gelation and cross-linking gave uniformly sized chitosan nanoparticles with an average diameter of 92 nm, while the cross-linking without ionic gelation produced spindly chitosan particles with an average length of 943 nm and width of 188 nm.

Keywords: Chitosan; Ultrafine particles; Microemulsion

1. Introduction

Chitosan, a β-(1,4)-linked polysaccharide of D-glucosamine, is a natural, linear and cationic biopolymer obtained from deacetylation of chitin, which is the second abundant biopolymer after cellulose and originates from shells of crustaceans such as crabs, prawns, lobsters, the cuticle of insects and the cell walls of fungi. Based on its many favorable characteristics, such as low toxicity (Tharanathan & Kittur, 2003), hydrophilicity, biodegradability (Chellat et al., 2000) and biocompatibility, chitosan has many biomedical and pharmaceutical applications including wound healing (Yusof, Lim, & Khor, 2001), antacid and antiulcer activities (Ito, Ban, & Ishihara, 2000), as a vehicle for tablets (Illum, 1998), a granulating agent (Ilango, Kavimani, Premila, Nair, & Jayakar, 1997), and a drug carrier (Lee, Kim, Kwon, & Jeong, 2000; Ruel-Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002) or a gene carrier (Roy, Mao, Huang, & Leong, 1999; Kim et al., 2001; Koping-Hoggard et al., 2001). Besides, chitosan possesses mucoadhesive properties (Shimoda, Onishi, & Machida, 2001; Kockisch, Rees, Young, Tsibouklis, & Smart, 2003) due to the electrostatic interaction between positively charged chitosan and negatively charged mucosal surfaces, and thus it can be used to enhance drug penetration across the nasal and buccal (Senel et al., 2000) mucosa. In addition, chitosan has a large number of amine groups which can serve as chelation sites (Ng, Cheung, & McKay, 2002), so it can absorb a number of metal ions (Kratochvil & Volesky, 1998) and can thus be utilized for the separation of metals.

Because of its excellent properties, chitosan has been used to form gel, fibers, sponges, films (Park et al., 1999), beads (Shu & Zhu, 2000), microspheres (Wang, Ma, & Su, 2005; Kosaraju, D'ath, & Lawrence, 2006) and nanoparticles (Janes, Calvo, & Alonso, 2001; Tang, Huang, & Lim, 2003; Qi, Xu, Jiang, Hu, & Zou, 2004). Since nanoparticles can be easily transported to different body sites and have large surface areas, a growing interest has been directed toward the exploration of chitosan nanoparticles. As the size distribution of chitosan nanoparticles plays an important role in their application in drug delivery, it is necessary to prepare uniformly sized chitosan nanoparticles. However, the size distribution of chitosan nanoparticles is very difficult to control. Wang et al. (2005) successfully controlled the size distribution of chitosan microspheres by membrane emulsification technique and prepared uniform-sized chitosan microspheres. In this paper, we prepared uniform-sized chitosan nanoparticles and microparticles by reverse microemulsion.

As an effective way to prepare ultrafine particles, reverse microemulsion (W/O) is a transparent, isotropic and thermodynamically stable synthesis medium. It consists of tiny aqueous

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droplets dispersed in a continuous oil phase and stabilized by surfactant molecules at the water/oil interface. These tiny droplets form a confined reaction medium to control the shape and size distribution of particles. They not only act as microreactors for the formation of ultrafine particles but also inhibit excessive aggregation of particles. Therefore, reverse microemulsion is a promising method for the preparation of nanosized or microsized particles.

2. Experimental

2.1. Materials

Water-soluble chitosan was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd. (Jinan, China), with a degree of deacetylation of 85.3% and MV (viscosity-average molecular weight) of about 60,000. It was purified by dissolving in distilled water and passing through a filter to remove any undissolved fraction of the as-purchased chitosan.

All chemicals used in the experiments were obtained from commercial sources as analytical reagents without further purification. Distilled water was used throughout the study.

2.2. Preparation of ultrafine chitosan particles by combining ionic gelation and cross-linking

Ultrafine chitosan particles were prepared by using reverse microemulsion, in which Triton X-100 was used as surfactant, octanol as co-surfactant, and cyclohexane as oil phase. The flow diagram for the formation of ultrafine chitosan particles is shown in Fig. 1.

Ultrafine chitosan particles were first prepared by ionic gelation according to the following typical procedure: 0.5 g of purified water-soluble chitosan was dissolved in 99.5 mL of distilled water to reach a final concentration of 0.5% (w/w). Six milliliter of the prepared chitosan solution and 6 mL of NaOH (0.1 M) solution containing a small amount of sodium dodecyl sulfate (SDS) were dropped respectively into two 24 mL of the mixtures of Triton X-100/octanol/ cyclohexane (7/8/9, v/v/v), which were subsequently sonicated and stirred for 30 min at room temperature to obtain homogenous and optically transparent chitosan microemulsion and SDS/NaOH microemulsion. Then the SDS/NaOH microemulsion was added drop-wise into the chitosan microemulsion till pH reached 10. Continue to sonicate and stir the microemulsion for 30 min to precipitate chitosan particles.

The resulting chitosan suspension was further modified by cross-linking with glutaraldehyde and formaldehyde. The method is as follows: 1.5 mL of a mixture of glutaraldehyde/formaldehyde (1/1, *n*/*n*) was slowly dropped into the above resulting suspension. While the reactivity of the cross-linker depends upon various factors, the crosslinking reaction was carried out at 40 °C for 2 h. To extract the ultrafine chitosan particles from reverse micelles and purify them from unreacted materials, the ultrafine particles suspensions were centrifuged at 5000 rpm for 30 min and the precipitate was then washed with acetone and ethanol for two or three times prior to drying under vacuum for 12 h to obtain ultrafine chitosan particles.

2.3. Preparation of ultrafine chitosan particles by cross-linking

Ultrafine chitosan particles were also prepared by cross-linking to compare with those by combining ionic gelation and cross-linking, the only difference being the lack of the ionic gelation step in the former method. The chemical crosslinking, washing and drying processes and other experimental conditions remained the same.

2.4. Characterization

The morphology of chitosan particles was observed by transmission electron microscopy (TEM) (TECNAI 20, Philips FEI, Netherlands). Samples were prepared for TEM analysis by placing a drop of chitosan particles suspension on a copper grid and quickly wicking away the solution with filter paper. Fourier transform infrared spectrometry (FT-IR) spectra were recorded with a Nicolet 60-SXB spectrometer in the

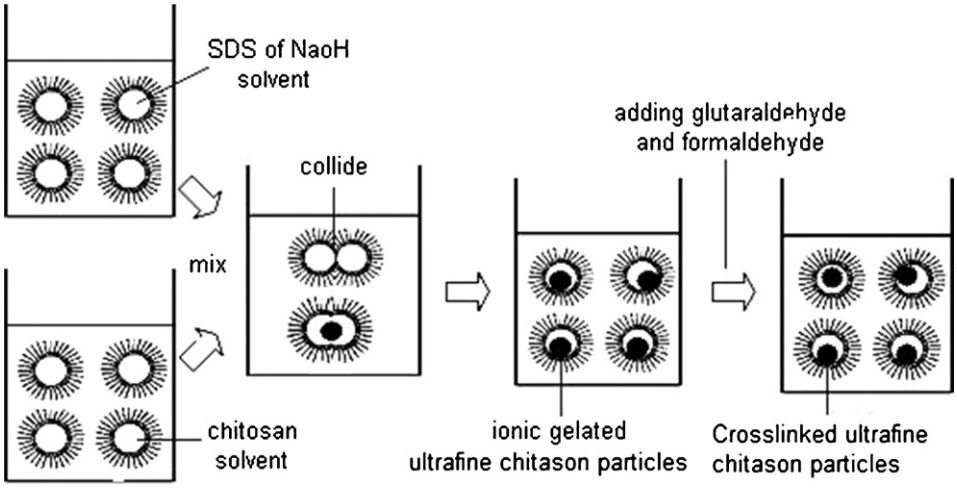

Fig. 1. Schematic illustration of the formation process of ultrafine chitosan particles.