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High efficient fabrication of chitosan micropowder by combination of gamma radiation and jet pulverization

Guozhong Wu*, Dewu Long, Shuiping Chen, Side Yao

Center for Applied Radiation and Materials, Shanghai Institute of Applied Physics, Chinese Academy of Science, CAS, P.O. Box 800-204, Shanghai 201800, China

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Abstract

We describe here a novel approach to the high efficient fabrication of chitosan micropowder by combining radiation technique and jet pulverization. The radiation dose is at 100–300 kGy. Size of microparticles in ethanol dispersion determined by dynamic laser scattering (DLS) is in the range of 5–20 μm, depending on the radiation dose and degree of deacetylation (DD). However, Scanning electron micrography (SEM) measurement shows a size range of 0.1–10 μm. FTIR spectra indicate that chemical structure of the micropowder is retained, while the decomposition temperature measured decreases slightly as compared to the control.

Keywords: Radiation; Jet pulverization; Micropowder; Chitosan

1. Introduction

Due to their excellent biomedical properties (e.g. inhibition of infection, acceleration of wound healing, antibacterial property, and even the suppression of some tumors), chitosan and its derivatives have attracted much interest in recent years. However, poor solubility of chitosan in water or polar solvent is the often-cited disadvantage in application. For that reason, chitosan is usually dissolved in aqueous acidic solution or degraded into oligomers with very low molecular weight. Numerous researchers have tried to modify the chemical structure of chitosan so that it is soluble in neutral/alkaline aqueous solution. Sulfonation, carboxymethylation, and N-quaternation are effective measures for that purpose. However, when fabricated into micro- or nanoparticles, chitosan may find use in a wide range of applications including cosmetics, food additives, textile industry, etc., since fine particles can be directly dispersed in water and viscous liquid without the use of acid. Chitosan microspheres and nanoparticles can also be used as carriers for drugs and proteins (Aksungur, Sungur, Unal, & Aksungur, 2004; Calvo, Vila-Jato, & Alonso, 1997a; De-Campos, Sanchez, & Alonso, 2001; Kumar, 2000; Ma, Yeoh, & Lim, 2002).

Many efforts have been devoted to preparing chitosan based micro- or nanoparticles. For instance, chitosan nanoparticles are prepared in diluted solution by ionic crosslinking using tripolyphosphate (TPP) (Janes & Alonso, 2003; Shiraishi, Imai, & Otagiri, 1993), sulfate (Berthold, Cremer, & Kreuter, 1996), or other inorganic anions. However, the efficiency of this approach is limited since the applied chitosan concentration is very low. Chitosan beads in the size of 0.5–0.710 mm are also prepared by TPP crosslinking at relatively high chitosan solutions (Ko, Park, Hwang, Park, & Lee, 2002). The advantage of ionic crosslinking is the lower toxicity than chemical crosslinking using glutaraldehyde (Banerjee, Mitra, Singh, Sharma, & Maitra, 2002), ethylene glycol diglycidyl ether (Mi, Shyu, Chen, & Schoung, 1999). Copolymers of chitosan with biocompatible polymer chains such as poly(acrylic acid) (Hu et al., 2002) and polyethylene oxide (Calvo, Remunan-Lopez, Vila-Jato, & Alonso, 1997b) are also applied for preparing hydrophilic nanoparticles by cross-linking. In addition, chitosan microparticles can also be

* Corresponding author. Tel./fax: +86 21 59558905.
E-mail address: wuguozhong@sinap.ac.cn (G. Wu).

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prepared by a combination of mechanic grinding and spray-drying process (Twu, Huang, Chang, & Wang, 2003), or by the joint action of high pressure and shear deformations in a two-screw extruder (Rogovina, Akopova, & Virkhoreva, 1998), which results in relative larger particle size.

Chitosan is known as a polymer of radiation degradation type, its molecular weight can be adjusted by varying the irradiation dose (Hai, Diep, Nagasawa, Yoshii, & Kume, 2003; Rosiak, Ulanski, Kucharska, Dutkiewicz, & Judkiewicz, 1992; Ulanski & Rosiak, 1992; Zhao, Zhong, Yu, Zhang, & Sun, 1993. Compared to chemical and enzymatic degradation methods, radiation is a simple and labour-saving method. Radiation degraded chitosan has excellent bioactivity and other novel biological properties (Matsuhashi & Kume, 1997; Tham et al., 2001. In this work, chitosan microparticles were high efficiently fabricated by jet pulverization of the irradiated chitosan powder. Effects of radiation dose on particle size and other properties were investigated.

2. Experimental

Commercial chitosan powder (~80 meshes) purchased from Yuhuan Biocompany, of China was used as received. A sample of 5.0 kg chitosan was irradiated in air by γ-ray to a dose of 100–300 kGy (dose rate: 6.5 Gy/min) at the ^{60}Co source of Shanghai Institute of Applied Physics. Micropowder was fabricated by jet pulverizing the irradiated sample with head pressure of 8.5 kg/cm^2.

Particle size and size distribution of micropowder were analyzed by laser dynamic scattering (DLS) by AccuSizer of PSS (USA) in ethanol dispersion and by JL-1188 system of Chendu Jingxin Co. (China) in the solid state, respectively. Morphology of particle was measured by scan electron micrograph (SEM). FTIR spectra of chitosan were taken in KBr pellets to check the radiation effect on chemical structure, using Perkin-Elmer FT-IR spectrophotometer instrument. Differential scanning calorimeter (DSC) was performed by a DCS Mettler model TA822e. Samples of 10 mg were scanned in sealed aluminum pans under N_2 atmosphere with a heating rate of 10 °C/min. To avoid the influence of moisture, the sample was kept at 80 °C for 15 min in the course of heating.

3. Results and discussion

3.1. Fabrication of micropowder

In analogy with cellulose and starch, chitosan undergoes degradation upon radiation. Radiation degradation mechanism of chitosan in aqueous solution as well as in the solid state has been previously studied. In aqueous solution, H abstraction by OH˙ radicals generated from water radiolysis leads to the formation of radicals localized on various carbon atoms and rearrangements of radicals localized on C_1, C_4 and C_5 carbons cause the scission of 1–4 glycosidic bond, resulting in the scission of chitosan polymer chain. In the solid state, radiation induced main chain scission results in the decrease of molecular weight. Radiation chemical yields of chain scission in the solid state are reported to be 0.87 μmol/J in vacuum, 1.07 μmol/J in air and 1.26 μmol/J in oxygen (Rosiak et al., 1992). Although G(scission) in the solid state is smaller than in the aqueous solution, irradiation in the solid state is favorable from the economic standpoint since the aqueous concentration is low and the drying requires additional energy.

For radiation degraded chitosan powder, the molecular weight is decreased as a result of the main chain scission. Therefore, it is possible to fabricate the irradiated chitosan into micropowder by jet pulverization, which is a powerful method for the fabrication of a variety of micropowders. The scheme is illustrated in Fig. 1.

Fig. 2 shows SEM of micropowders prepared by jet pulverization of chitosan powder with different doses. It is shown that chitosan microparticles have irregular shape and they may contain smaller particles in the aggregated form. For the chitosan with DD 96%, particle size decreases obviously with increasing the radiation dose. The particle sizes are 15–25 μm at 100 kGy, 5–15 μm at 200 kGy, and <5 μm at 300 kGy. This indicates a high radiation dose for the fabrication of micropowder with diameters less than 10 μm. However, as shown in Fig. 2(D), for chitosan with lower DD (76%), submicronized powder is obtained at 200 kGy under identical jet pulverizing conditions. It implies that lower DD favors the fabrication of micropowder with smaller size at the same radiation dose.

To further prove the effect of DD on particle size, we tried to prepare chitosan micropowder in the additional experiment by high speed shearing (~12,000 rpm) of irradiated sample in neutral water. Water is used as

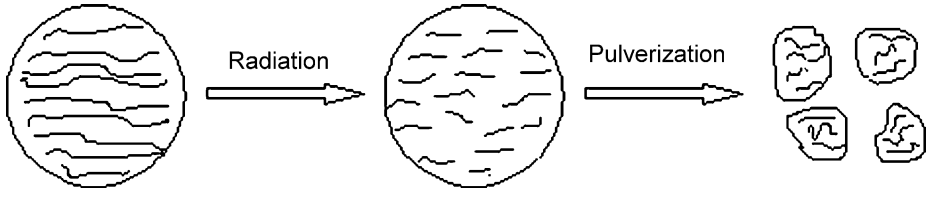

Fig. 1. Schematic diagram of the fabrication of chitosan micropowder.

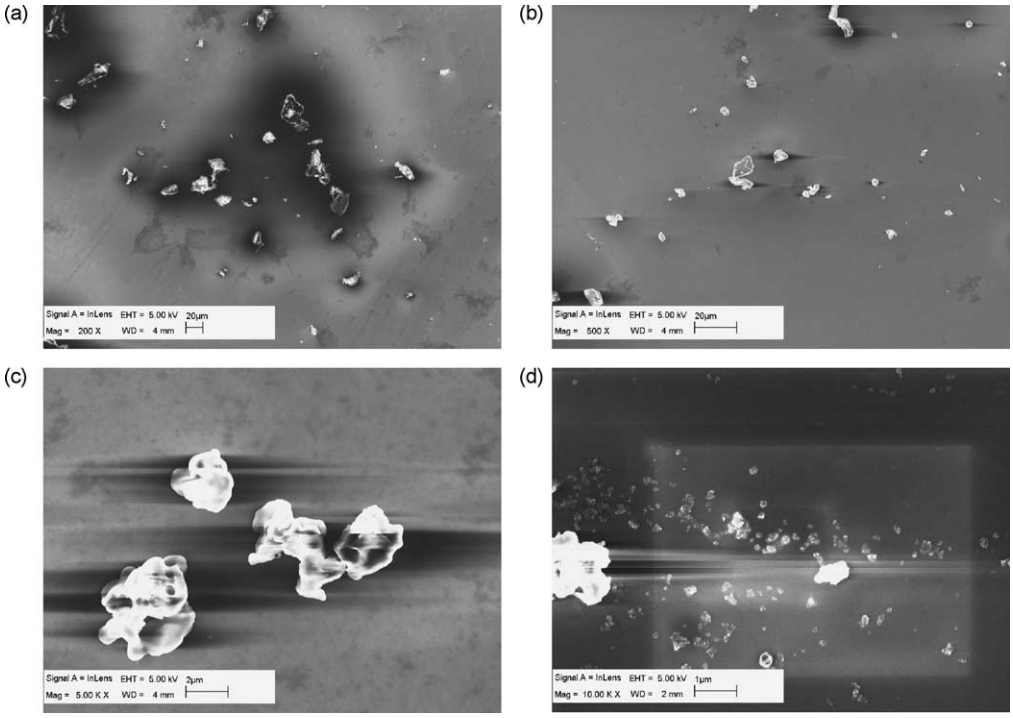

Fig. 2. SEM of chitosan micropowder fabricated by jet pulverization. (a→c): DD 96%; 100, 200, 300 kGy; (d): DD 76%, 200 kGy. Magnification: (a) ×200; (b) ×500; (c) ×5000; (d) ×10,000.

a dispersion medium since chitosan is water insoluble. Fig. 3 shows SEM of the obtained micropowder of chitosan with DD of 76% at 200 and 300 kGy, respectively. It clearly shows that the microparticles obtained at 200 kGy are relatively uniform in size and their sizes are ∼0.2 μm. At 300 kGy, particle sizes become smaller but the particle shape is irregular, probably due to the formation of aqueous soluble chitosan oilgomers. For chitosan with DD of 96%,

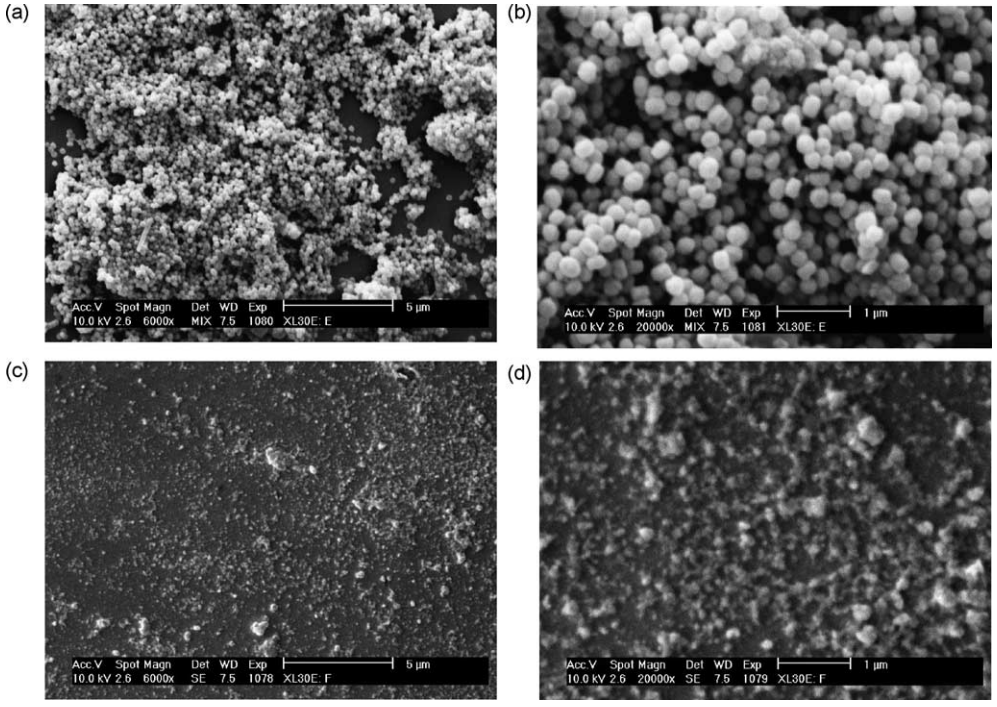

Fig. 3. SEM of chitosan (DD 76%) micropowder prepared by high-speed shearing (12000 rpm) in water. (a,b): 200 kGy; (c,d): 300 kGy. Magnification: (a,c), ×6000; (b,d), ×20,000.

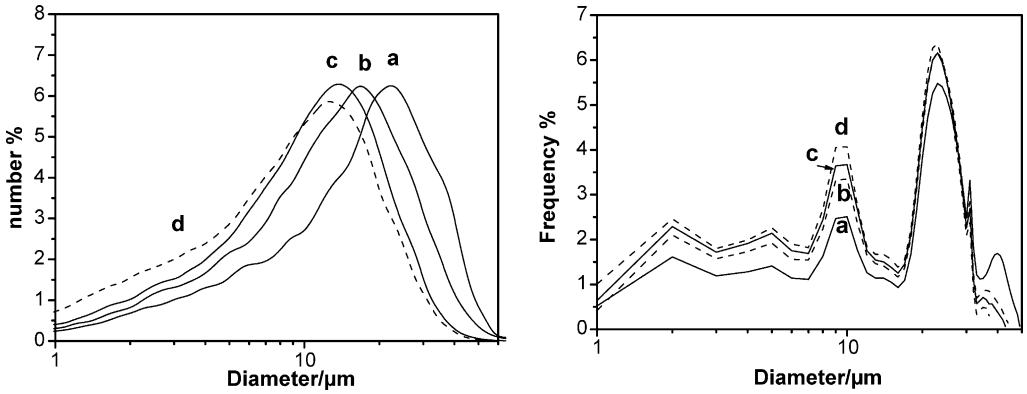

Fig. 4. Size distribution of chitosan micropowder measured in ethanol dispersion (left) and directly in the solid state (right). Samples are the same as shown in Fig. 2.

however, we failed to obtain micropowder by high speed shearing. This may be due to the difference in brittleness between the two samples applied.

Although microparticles can also be fabricated in water by high speed shearing for chitosan with lower DD, additional processes (e.g. drying) are required to obtain micropowder. We consider that jet pulverization is the better choice and it is suitable for samples with any DD.

3.2. Size distribution of micropowder

As shown in Fig. 3, the mean particle size decreases gradually with increasing the dose. For larger microparticles, the measured sizes are consistent with the observation by SEM. For smaller particles, however, the size measured by DLS is significantly larger. This can be ascribed to the irregular particle shape and aggregation of particle in ethanol dispersion. We also found that the measured size becomes smaller with dilution but the error is large.

The size distribution was further determined in the solid state for comparison. As also shown in Fig. 4, the particle size also decreases with radiation dose, but two peaks are always observed for all the samples. The peak at higher diameter size is likely due to the aggregation of particle. This measurement is in reasonable agreement that observed in the ethanol dispersion, although the pattern of size distribution is quite different.

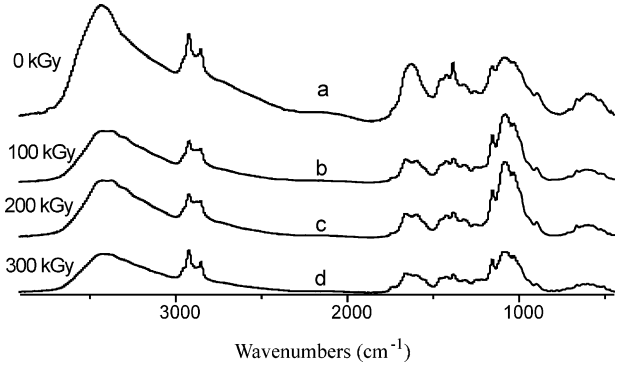

Fig. 5. FT-IR spectra of the control and prepared chitosan micropowder. Samples numbers are the same as shown in Fig. 2.

3.3. FTIR and DSC measurement

Properties of the chitosan micropowder were measured by FTIR and DSC and compared with those of the virgin sample. The main purpose is to know whether irradiation and jet pulverization cause a serious loss of amino groups ($-NH_2$) on pyranose ring, since the amino group plays an important role in chitosan's bioactivity. As clearly shown in Fig. 5, FTIR spectra indicate no evident difference for chitosan samples. The DD can be calculated by the equation (Domard & Rinaudo, 1983)

$$DD = (A_{1655}\ \mathrm{cm}^{-1}/A_{3450}\ \mathrm{cm}^{-1}) \times 100/1.33$$

where A_{1655} and A_{3450} denote the absorption at 1655 cm^{-1} and 3450 cm^{-1}, respectively, corresponding to C=O vibration and OH stretching.

The calculated DD values are 96.8, 96.9, 97.1 and 96.6% for the control and micropowders with radiation doses of 100, 200, and 300 kGy, respectively. This indicates that

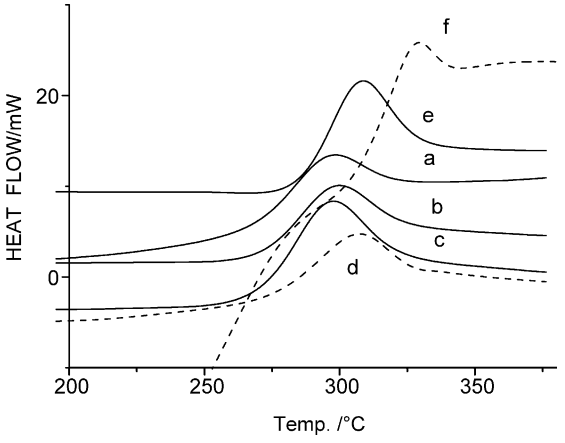

Fig. 6. DSC thermograms of chitosan micropowder and control. (a→d): same samples as shown in Fig. 2; (e): control, DD 96%, (f): control, DD 76%.

radiation and jet pulverization lead to the cleavage of the only glycosidic bond keeping the amino groups intact. This finding is in agreement with the recent report by Yoksan, Akashi, Miyata, and Chirachanchair (2004), who observed that low-molecular-weight chitosan prepared by gamma radiation retains its functionality as compared to the degradation by chemical oxidation or acid/base hydrolysis.

Fig. 6 shows DSC thermograms the control and chitosan micropowder. As can be seen, the decomposition peak temperature (T_p) decreases with increase of radiation dose or decrease of particle size. For chitosan with DD of 96%, T_p decreases from 308 to 297 °C with increasing the dose from zero to 100 kGy. However, T_p keeps almost constant at doses of 100–300 kGy. For chitosan with DD of 76%, T_p decreases from 330 to 306 °C with increasing the dose from zero to 200 kGy. The observed decrease of T_p can be explained by the decrease in molecular weight due to radiation and jet pulverization, because it has been found (Kittur, Prashanth, & Tharanathan, 2002) that T_p decreases with increase in DD and decrease in molecular weight.

4. Conclusion

Chitosan micropowder was successfully fabricated by combining techniques of radiation and jet pulverization. The particle size is dependent on the radiation dose and DD. Although decomposition peak temperature of micropowder is decreased, the content of amino groups on the pyranose ring remains unaffected, which is of great importance in application. This approach offers the possibility to fabricate chitosan micropowder in large scale, facilitating its use in a wide range of applications.

Acknowledgements

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