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Formulation and characterization of a plasma sterilized, pharmaceutical grade chitosan powder

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ABSTRACT

Chitosan has great potential as a pharmaceutical excipient. In this study, chitosan flake was micronized using cryo-ball and cryo-jet milling and subsequently sterilized with nitrogen plasma. Micronized chitosan was characterized by laser diffraction, scanning electron microscopy (SEM), conductometric titration, viscometry, loss on drying, FTIR, and limulus amebocyte lysate (LAL) assays. Cryo-jet milling produced mean particle size of 16.05 μm, 44% smaller than cryo-ball milling. Cryomilled chitosan demonstrated increased hygroscopicity, but reduced molecular weight and degree of deacetylation (DD). SEM imaging showed highly irregular shapes. FTIR showed changes consistent with reduced DD and an unexplained shift at 1100 cm^{-1}. Plasma treated chitosan was sterile with <2.5 EU/g after low-pressure plasma and <1.3 EU/g after atmospheric pressure plasma treatment. Plasma treatment decreased the reduced viscosity of chitosan flake and powder, with a greater effect on powder. In conclusion, pharmaceutical grade, sterile chitosan powder was produced with cryo-jet milling and plasma sterilization.

1. Introduction

Chitosan (CS) in the presence of moisture is a soft, ductile biopolymer with great potential as a biomedical material, especially as an excipient and immune adjuvant. CS is obtained commercially by deacetylating chitin extracted from either crustacean shells via chemical extraction or fungi via enzymatic extraction and consists of β-1,4-linked 2-amino-2-deoxy-D-gluocopyranose and *N*-acetamido-2-deoxy-D-glucopyranose moieties that are randomly distributed throughout the polymer chain. Since chitin is the second most abundant biopolymer on Earth and crustacean shell waste generated by the seafood industry is an environmental problem in coastal areas, CS is a widely available, green, and economical biomaterial. Chitin becomes CS once at least 50% of the D-glucosamine moieties in the polymer chain are deacetylated (Pillai, Paul, & Sharma, 2009). However, there is disagreement over the naming of chitin and CS based on degree of deacetylation, which has resulted in a proposal for naming chitin and CS based on solubility in acetic acid (Badawy & Rabea, 2011; Kumar, 2000). CS is soluble in aqueous acetic acid, whereas chitin is not.

As an excipient, CS enhances drug penetration through tissues and epithelial barriers by loosening gap junctions, maintains drug in the area of interest through bioadhesion between cationic amino groups of CS and anionic tissues, and controls drug release over time by keeping drugs bound until physical degradation (Artursson, Lindmark, Davis, & Illum, 1994; Dodane, Amin Khan, & Merwin, 1999; Jameela, Misra, & Jayakrishnan, 1994; Kristl, Šmid-Korbar, Štruc, Schara, & Rupprecht, 1993; Luessen et al., 1996; Nordtveit, Vårum, & Smidsrød, 1994; Schipper, Vårum, & Artursson, 1996; Takeuchi, Yamamoto, Niwa, Hino, & Kawashima, 1996; van der Lubben, Verhoef, van Aelst, Borchard, & Junginger, 2001). The biological functions of CS are dependent on its physical properties, including molecular weight (MW), degree of deacetylation (DD), salt form, and pH at which it is used (Dodane & Vilivalam, 1998;

Abbreviations: CS, chitosan; NtNP, non-thermal nitrogen plasma; ApP, atmospheric pressure plasma; LpP, low-pressure plasma; DD, degree of deacetylation.

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Kumirska, Weinhold, Thöming, & Stepnowski, 2011). This physical-functional relationship necessitates careful characterization of CS formulations. Since powder and hydrogel forms of CS – especially those composed of particles in the low micron range – have been identified as the ideal form of CS for pharmaceutical applications and micronization of materials is a common process in pharmaceutical manufacturing, the goal of the present study was to formulate and characterize a pharmaceutical grade CS powder that can also serve as a precursor to a hydrogel when dissolved in dilute acids (van der Lubben et al., 2001).

A powder form of CS was also desired to enhance the effectiveness of a novel sterilization method for CS based on non-thermal nitrogen gas plasma (NtNP) (de Oliveira Cardoso Macedoet al., 2013). Plasma is considered the fourth state of matter and consists of an ionized gas that has emergent properties the gas alone does not, such as magnetism and conductivity. Use of NtNP for sterilization of CS is necessitated by the fact that conventional sterilization methods like dry/wet heat, radiation, and chemical sterilants cause caramelization of the polysaccharide, chain scissions, and/or may leave residual toxic residues in the material (de Oliveira Cardoso Macedo et al., 2013; Franca et al., 2013; Lim, Khor, & Koo, 1998; Lim, Khor, & Ling, 1999; Marreco, da Luz Moreira, Genari, & Moraes, 2004; Norzita et al., 2013; Rao & Sharma, 1995; Rosiak, Ulański, Kucharska, Dutkiewicz, & Judkiewicz, 1992; San Juan et al., 2012). These physicochemical changes result in changes in the biological properties of CS making these methods non-optimal for sterilizing CS. Since NtNP is a surface treatment, it benefits from a large surface to volume ratio, which is accomplished when a material is micronized.

Knowledge on micronizing hard and crystalline materials is extensive, but lacking on softer materials. It is vital to characterize particle size and size distribution of powders since these properties influence flowability, dissolution, release kinetics, and more (Koennings, Sapin, Blunk, Menei, & Goepferich, 2007; Miranda, Millán, & Caraballo, 2007; Mullarney & Leyva, 2009). Reports in the literature of techniques for generating micronized CS powders are especially sparse. Techniques for micronizing CS that have been reported include dense gas anti-solvent precipitation, supercritical-assisted atomization, microsphere precipitation, high speed planetary ball mill, and ultrafine milling (Chien, Li, Lee, & Chen, 2013; Gimeno et al., 2006; Reverchon & Antonacci, 2006; Yao, Peng, Yin, Xu, & Goosen, 1995; Zhang, Zhang, Jiang & Xia, 2012; Zhang, Zhang, & Xia, 2014). We identified cryomilling as an optimal method for generating a CS powder based on previous studies that show it preserves functional properties of proteins and starches by reducing the energy input needed to fractionate particles into smaller sizes (Dhital, Shrestha, Flanagan, Hasjim, & Gidley, 2011; Ehmer, 2010; Tran et al., 2011). Additionally, cryomilling overcomes the soft ductile nature of CS, which reduces the effectiveness of traditional milling techniques in micronizing soft materials like CS (Garmise et al., 2006; Saleem & Smyth, 2010).

In the present study, two cryomilling techniques were tested, cryo-jet and cryo-ball milling. Ball and jet mills were chosen for this study since they are the only milling machines commonly used to reduce particles to 5 μm or less in dry conditions (Vatsaraj et al., 2003). Both ball and jet mills are thought to reduce particle sizes using the same mechanism(s), which is by breaking particles along cracks or fractures that already exist at the micro- or nanoscale. Although jet milling is the most commonly used technique for producing particles in the lower micrometer range and is the gold standard for manufacture of inhalable particles of small molecular drugs, this study is the first reported use of cryo-jet milling for micronizing CS (Ehmer, 2010). Once the optimal CS powder formulation was identified, defined as the micronized powder with the smallest mean particle size, it was sterilized with NtNP to form a sterile, pharmaceutical grade CS powder. Physicochemical properties of the CS were characterized before and after both milling and NtNP sterilization.

2. Materials and methods

2.1. Reagents

All chemical reagents including glacial acetic acid, lactic acid, sodium acetate, hydrochloric acid, sodium hydroxide, and tryptic soy agar were obtained from Sigma–Aldrich or Fisher Scientific and were of analytical grade.

2.2. Cryomilling

CS derived from crab shells in the form of 1–10 mm flakes was obtained from Scion Biomedical, Inc. (Miami, FL). CS flakes were filtered with fine mesh to remove flakes >8 mm in diameter and approximately 30 g of filtered CS flake was placed in a 25 mL zirconium oxide jar. Six milling balls made of either zirconium oxide (10 mm diameter) or stainless steel (5 mm) were added to the jar and the CS flakes were subsequently milled under cooling with liquid nitrogen at 30 Hz for up to 30 min in the Retsch CryoMill system (Verder Scientific, Inc., Newtown, PA). For cryo-jet milling, CS flakes were filtered, as described above, to remove flakes >8 mm, 470 g of filtered CS was placed in the Micron-Master jet pulverization system (The Jet Pulverization Co., Inc., Moorestown, NJ), cooled with liquid nitrogen, and milled with a jet stream of liquid nitrogen for 30 min.

2.3. Particle sizing

Particle size analyses were performed on powders by suspending CS in water and measuring particle size with a Horiba LA-930 laser diffraction analyzer (HORIBA Instruments, Inc., Irvine, CA).

2.4. Degree of deacetylation

Degree of deacetylation (DD) of both CS flake and powder was determined by conductometric titration. Conductometric titration was performed by dissolving dried CS sample of known mass (about 0.100 g) into 10 mL of 0.1 N hydrochloric acid (HCl) then 90 mL of distilled water. The CS solution was then titrated with a standard 0.1 N sodium hydroxide (NaOH) solution using a 10 mL buret while the solution conductivity was monitored as a function of the volume of NaOH added with an Orion Benchtop Conductivity Meter (Model 162) equipped with an Orion Conductivity Cell (Model 013030) (Raymond, Morin, & Marchessault, 1993). During the titration, the temperature of the solution was kept constant (30 °C) by using a water bath since the conductivity is a function of temperature. In a typical conductometric titration curve, there are two deflection points. The first deflection point corresponds to the neutralization of excess H^+ ions of the strong acid, HCl. After all excess H^+ ions are neutralized, then the neutralization of the weak acid, the ammonium salt in CS, starts. After the ammonium is completely neutralized, the conductivity again goes up with a higher value of slope due to the excess of OH^- ions of NaOH added, which is the second deflection point. Thus, the range between the first and the second deflection points corresponds to the neutralization of the protonated amine groups of CS. As a result, the number of moles of NaOH used between the first and second deflection points equals the number of moles of amine groups of the CS sample. The %DD was calculated by the following equation:

$$\%DD = \frac{(v_2 - v_1) \times M_{NaOH}\left(\frac{mol}{L}\right) \times \frac{161.16g}{mol}}{\text{Mass of chitosan sample } (g)} \times 100 \quad (1)$$