SUPERCRITICAL ANTI-SOLVENT AND ATOMIZATION PROCESSES FOR THE ELABORATION OF NANOPARTICLES OF CHITOSAN

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ABSTRACT

Among several other biobased, biocompatible and biodegradable polymers, chitosan appears as a good candidate for human health applications. It is an abundant polysaccharide and shows antibacterial and cytocompatible properties.
In this study, we developed two supercritical processes aimed at generating chitosan nanoparticles for their further incorporation in biobased films by extrusion with potential applications in packaging and biomedical devices.
As a first step, chitosan nanoparticles were generated using two supercritical CO_2 based processes, in order to minimize the use of organic solvent: (1) Supercritical AntiSolvent process: CO_2 acts as an antisolvent towards an acetic acid aqueous solution of dissolved chitosan in which ethanol was added to enhance the antisolvent effect. The reciprocal miscibility of CO_2 with the solvents induces chitosan supersaturation, which in turn, causes the crystallization of the particles. This process led to chitosan nanoparticles generation with an average size of 378. (2) SCASA: pressurized CO_2 is dissolved into water to lower the pH. This acidification allows the chitosan to be dissolved and the resultant solution is sprayed into a hot air stream. This new process allowed the generation of dried chitosan nanoparticles with a median diameter of 400 nm with less than 10 % particles larger than 1μm.

INTRODUCTION

Micro-scaled and nano-scaled particulate systems become increasingly important in several domains especially for drug delivery applications [1]. Research on biosourced materials for the drug delivery is intensive and among several other biobased, biocompatible and biodegradable polymers, chitosan appears as a good candidate for human health applications [2].
Chitosan is an amino-polysaccharide made of poly β (1-4) D-glucosamine and N-acetyl-D-glucosamine obtained by an alkali treatment of chitin, the major component of crustacean shells and fungi cell walls. It has many assets such as nontoxicity, cytocompatibility and antimicrobial activity [3].
Several studies have been conducted to generate chitosan particles from acetic acid aqueous solutions mainly by spray-drying, emulsion-crosslinking, coacervation-precipitation [4] and more unusually, using carbon dioxide (Sc-CO_2) as expansion agent [5, 6].

In this study, we developed two supercritical processes aimed at generating chitosan nanoparticles for their further incorporation in biobased films by extrusion with potential applications in packaging and biomedical devices.

MATERIALS AND METHODS

Commercial chitosan extracted from shrimp shells was purchased from France Chitine (France). Its viscosity and acetylation degree (DA), given by the supplier, are respectively about 50 mPa.s for a 1% (w/w) chitosan/acetic acid solution at 298 K and 10%. The viscosity average molecular weight M_v was found to be 62 kDa using Mark-Houwink Sakurada law and Huggins equation. When chitosan is put into demineralized water, the pH of the resulting dispersion is around 13 due to alkaline residue of the deacetylation process. Acetic acid (90%) and potassium hydroxide were purchased from Prolabo (France); Ethanol (96%) is from VWR (France). CO_2 (purity 99.995%) was supplied by Air Liquide (France) and used without any further purification.

Chitosan nanoparticles were generated using two supercritical CO_2 based processes, in order to minimize the use of organic solvents:

(1) Supercritical AntiSolvent process (SAS): CO_2 acts as an antisolvent towards an acetic acid aqueous solution of dissolved chitosan in which ethanol was added to enhance the antisolvent effect. The reciprocal miscibility of CO_2 with the solvents induces chitosan supersaturation, which in turn, causes the crystallization of the particles.

Beforehand, a potassium hydroxide alkaline solution is placed inside the crystallization vessel to receive and stop further evolution of the generated particles by neutralizing any residual acidity remaining on the particles and induced by CO_2. CO_2 loaded with solvents is recycled in the separators. At the end of the injection, the vessel is depressurized and the particles in suspension are collected and washed with demineralized water. The suspension is then freeze-dried to collect dry particles.

(2) Sc-CO_2 Assisted Solubilization and Atomization (SCASA): chitosan powder is first dispersed in distilled water and placed inside the vessel under stirring. The vessel is then filled and pressurized with CO_2. Under chosen high pressure and moderate temperature, the acidifying power of the CO_2 causes the dissolution of the chitosan powder. The solution is then sprayed in a fluidized bed and carried into a hot air stream to be dried. During the depressurization, CO_2 turns back to its gaseous state inducing the crystallization of chitosan from droplets. After spraying, particles are collected in the filters.

RESULTS

Using SAS process, two different generated morphologies were observed depending on the concentration of the alkaline solution: in the case of a concentrated potassium hydroxide solution (2% w/w), nanoscaled particles with an average diameter of 378 ± 13 nm were observed in a porous network of chitosan (Figure 1(c)), whereas a less concentrated alkaline solution (1%w/w) contained a better defined nanoparticles (Figure 1(b)) with an average diameter of 820 ± 19 nm. The strong influence of the chemistry of the systems involved, in particular the pH, seem to alter the morphology of the polymer material. Nevertheless, in both cases, the generated particles have different morphologies than the commercial chitosan

particles (Figure 1(a)). This confirms the ability of the SAS process to generate nanoparticles of chitosan.

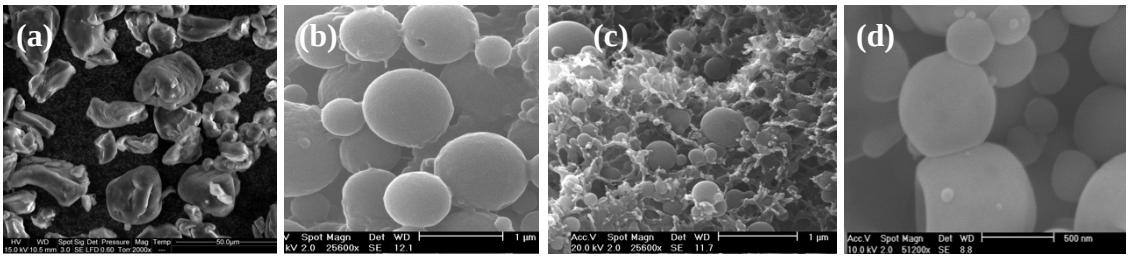

Figure 1: *SEM photographs of commercial chitosan (a) and chitosan particles generated by SAS process for two potassium hydroxide concentrations (1% (b) and 2% (c)) and by SCASA process (d)*

Using SCASA process, different chitosan suspensions were tested and dried particles were directly collected in the filters: as shown in Figure 1(d), these particles are mainly spherical. Some of them are hollow-shaped, which could be explained by the desorption of the CO_2 during the solidification of chitosan spheres. The variation of chitosan concentration did not seem to significantly affect the particle generation nor the particle size. Over 500 measured particle diameters, generated particles have a bi-modal size distribution: nanoscaled particles for the most part with a median diameter of 390 nm (less than 10% of the measured particles larger than 1 µm) and microscaled particles (median diameter around 20µm) mainly due to the agglomeration of nanoscaled particles.

The XRD analysis showed that both processes allowed the generation of repeatable partially amorphized structures compared to the commercial chitosan.

Acetylation degree was evaluated for both processes using IR spectroscopy and found to be around 20%. No significant difference could be assigned to the process.

CONCLUSION

During this work, a method for generating chitosan particles by anti-solvent effect using the sc-CO_2 has been designed and tested. Nanometer scaled spherical particles of a mean diameter of 378 ± 13 nm were produced with a crystallinity differing from the commercial chitosan. Morphology and aspect strongly depend on the recovery medium that seems to have an influence on the organization of polymer chains. In this context, a further study on the stability of the particles in suspension should be performed.

A new sc-CO_2 assisted solubilization and atomization process called SCASA was setup. It is simple, organic solvent-free and repeatable. It allowed the generation of near-spherical and spherical shaped particles mainly, without being destabilized by small parameter variations. However, two particle size populations were noticed: nanometric scaled particles of a median diameter of 390 nm and micrometric scaled particles of a mean diameter around 20 µm formed by the agglomeration of the first with a presence of individual spherical particles larger than 1 µm by less than 10 % in number of the total measurements. Many of the particles seem hollow-shaped probably due to the CO_2 desorption before a complete solidification of the

particles. A further step regarding this process will consist in optimizing the process parameters to have homogeneous sized particles with a high yield.

Subsequently, we will disperse these particles by melt compounding in a biopolymer matrix to produce a composite for biomedical applications.

REFERENCES

[1] WILCZEWSKA Z., A., NIEMIROWICZ, K., MARKIEWICZ, K.H, CAR, H. *Review - Nanoparticles as drug delivery systems.* Pharmacological Reports, Vol. 64, **2012**, p. 1020

[2] HARISH PRASHANTH, K.V., THARANATHAN, R.N. *Chitin/chitosan: modifications and their unlimited application potential- an overview*. Trends in Food Science & Technology, Vol. 18, **2007**, p. 117

[3] BADOT, P.M., CRINI, G., GUIBAL, E. *Chitine et chitosane: du biopolymère à l'application.* Presses Universitaires de Franche-Comté, 2009, Franche-Comté.

[4] DASH, M., CHIELLINI, F., OTTENBRITE, R.M., CHIELLINI, E. *Chitosan – A versatile semi-synthetic polymer in biomedical applications.* Progress in Polymer Science, Vol. 36, **2011**, p. 981

[5] REVERCHON, E., ANTONACCI, A. *Chitosan microparticles production by supercritical fluid processing.* Industrial & Engineering Chemistry Research, Vol. 45, **2006**, p. 5722

[6] SHEN, Y.B., DU, Z., WANG, Q., GUAN, Y.X., YAO, S.J. *Preparation of chitosan microparticles with diverse molecular weights using supercritical fluid assisted atomization introduced by hydrodynamic cavitation mixer.* Powder technology, Vol. 254, **2014**, p. 416