

DEVELOPMENT OF NANOSTRUCTURED FILMS BASED ON PLA AND CHITOSAN NANOPARTICLES GENERATED BY SUPERCRITICAL CO₂ ASSISTED PROCESSES

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Keywords: Biocomposite, supercritical CO₂, chitosan nanoparticles, cast extrusion

Abstract

In this study, supercritical processes were set-up to generate chitosan nanoparticles that were incorporated in biobased PLA films produced by cast extrusion. Firstly, chitosan nanoparticles were generated using two CO₂ based processes: (1) Supercritical AntiSolvent process where CO₂ acts as an antisolvent towards an acetic acid aqueous solution. Nanoparticles with an average size of 378 nm were obtained. (2) Sc-CO₂ assisted solubilisation and atomization process where chitosan was dissolved in CO₂ acidified water followed by spray-drying. Dried nanoparticles with a median diameter of 400 nm were generated. These generated nanoparticles and other chitosan particles were dispersed in PLA (2.4% w/w) by cast extrusion using a micro-compounder. The morphology and properties of the resultant nanostructured composite films were studied. Particles dispersion and size distribution within the films were analysed by optical and scanning electron microscopy and showed a good dispersion of the particles with no agglomeration. Differential scanning calorimetry revealed a nucleating effect of the chitosan nanoparticles.

1. Introduction

Microstructured and nanostructured systems become increasingly important in various domains such as packaging and tissue engineering. Research on biobased materials for these applications is serious and one of the promising biobased polymers is Polylactic acid (PLA). It is a linear thermoplastic biopolyester obtained from renewable and abundant resources, mostly by fermentation of starchy materials (corn, potatoes...) [1]. It has several interesting properties such as its transparency, low toxicity, biocompatibility and degradability in biological environment however it is brittle and has high gas permeability [2]. Hence, different nanoparticulate fillers were added to PLA matrix to enhance some of its properties and to extend its applications, for instance, silicates [3, 4], such as montmorillonite [5], etc.

To our knowledge, no study has ever been carried out to evaluate the effect of chitosan nanoparticles on PLA films obtained by hot melt extrusion.

Chitosan is an amino-polysaccharide made of poly β (1-4) D-glucosamine and N-acetyl-D-glucosamine obtained by deacetylation in alkali conditions of chitin, the major component of crustacean shells and fungi cell walls. It has many assets – beside being biobased, biocompatible and biodegradable – such as nontoxicity, cytocompatibility and antimicrobial activity [6, 7].

Chitosane particles were usually generated from acetic acid aqueous solutions mainly by spray-drying, emulsion-crosslinking, coacervation-precipitation [8] and more unusually, using carbon dioxide (Sc-CO₂) as expansion agent [9, 10]. Bonilla et al. [11] studied the effect of 5% and 10% of ground chitosan microparticles (715 and 180 μm) on the physical and chemical properties and the antimicrobial activity of PLA films. They found that composite films were less rigid, less mechanically resistant and more permeable to water vapour than pure PLA film; no significant changes in the thermal behaviour were noticed but the crystallization temperature increased and an antimicrobial effect was observed.

In this study, we have developed two supercritical CO₂ assisted processes aimed at generating chitosan nanoparticles. These nanoparticles and other chitosan particles were incorporated in PLA films by cast extrusion using a micro-compounder. The morphology and properties of the resultant nanostructured composite films were studied. Particles dispersion and size distribution within the films were analysed by optical and scanning electron microscopy. Crystalline structures of the films were characterized by differential scanning calorimetry (DSC).

2. Experimental

2.1. Materials

Commercial chitosan extracted from shrimp shells was purchased from France Chitine (France). Its viscosity is about 50 mPa.s for a 1% (w/w) chitosan/acetic acid solution at 298 K and acetylation degree (DA) is 10%. The average viscometric 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 the presence of residual sodium hydroxide from the deacetylation process. Acetic acid (90%) and potassium hydroxide were purchased from Prolabo (France); Ethanol (96%) is from VWR (France). CO₂ (purity 99.995%) was supplied by Air Liquide (France) and used without any further purification. Film extrusion grade PLA (PLE003) in pellets (density of 1.25 g.cm⁻³, melting temperature T_m between 418 and 433 K) was purchased from NaturePlast (France).

2.2. Particle generation processes

Chitosan nanoparticles were generated using two supercritical CO₂ based processes, in order to minimize the use of organic solvents and by a nebulization process so as to compare the results:

(1) Supercritical AntiSolvent process (SAS): CO₂ 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 CO₂ with the solvents induces chitosan supersaturation, which in turn, causes the crystallization of the particles.

Beforehand, a potassium hydroxide alkaline solution was 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 CO₂. CO₂ 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 dried particles.

(2) Sc-CO₂ Assisted Solubilisation 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 CO₂. Under chosen high pressure and moderate temperature, the acidifying power of the CO₂ causes the dissolution of the chitosan powder. The solution is then sprayed in a fluidized bed and sprayed into a hot air stream to be dried. During the depressurization, CO₂ turns back to its gaseous state inducing the crystallization of chitosan from droplets. After spraying, dried particles are collected in the filters.

(3) Nebulization process: commercial chitosan powder is dissolved in acetic acid (1:1 w/w) aqueous solution under stirring at room temperature. The solution is then nebulized into a 1M NaOH solution under continuous stirring. Afterwards, the obtained suspension is washed with demineralized water several times then freeze-dried.

2.3. Film extrusion process

PLA pellets and all chitosan particles were dried at 353 K for 4 h. Pure PLA and chitosan/PLA (2.4 / 97.6% w/w) composite films were prepared by extrusion using a 15 cm³ twin-screw micro-compounder (DSM Xplorer micro-compounder, Geleen, The Netherlands). After several preliminary tests, a screw speed of 70 rpm, and mixing time of 5 min, a speed roll of 600 mm·min⁻¹ and a uniform temperature of 453 K were set-up to prevent thermal degradation of PLA. Afterwards, films were conditioned at room temperature (298 K). Film thickness was measured using a Palmer digital micrometer (IHM, France) to the nearest 0.001 µm. Six random positions in each film sample were considered. Film formulations were named as follows: "PLA", "Com : PLA", "Nebu : PLA", "Nano : PLA", where Com, Nebu and Nano stand respectively for the commercial chitosan, nebulized chitosan and nanoparticles chitosan generated by SCASA process.

2.4. Particles and films characterization

Particle size distribution and mean particle diameter in the suspensions were measured using a Nano Zetasizer (Malvern Instruments, France) by the evaluation of the Brownian diffusion coefficient. Dried chitosan particles were visualized with an Environmental Scanning Electron Microscope (ESEM XL30 FEG, FEI Philips, Netherlands). For each sample, over 500 particles from at least four pictures were analysed with a numerical caliper integrated in the image acquisition software in order to determine mean particle diameter and the particle size distribution weighted in number.

The dispersion of the different chitosan powders in PLA films was analysed by optical microscope (Leitz Laborlux 11 POL, Leitz,) coupled with a digital camera (PC-Mount 3 CDD colour video camera, JVC instructions, USA).

A differential scanning calorimeter (Diamond DSC, PerkinElmer, USA) was used to characterize the melting and crystallisation of the different composite films. Heating and cooling thermograms were obtained between 303 K and 453 K with a heating rate of 10 K·min⁻¹ in nitrogen gas stream of 20 cm³·min⁻¹.

3. Results and discussion

3.1. Size distribution of the different generated particles

Nebulization process allowed the generation of particles with flaky aspect and high porosity (Figure 1(a)) with a large particle size distribution. The obtained particles had a mean particle size of $39 \pm 23 \mu\text{m}$.

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 $378 \pm 13 \text{ nm}$ were observed in a porous network of chitosan (Figure 1(b)), whereas a less concentrated alkaline solution (1% w/w) led to better defined nanoparticles (Figure 1(c)) with an average diameter of $820 \pm 19 \text{ nm}$. The strong influence of the chemistry of the systems, in particular the pH, seems to alter the morphology of the particles. Nevertheless, in both cases, the generated particles have different morphologies and size than the commercial chitosan particles (Figure 1(d)). This confirms the ability of the SAS process to generate nanoparticles of chitosan.

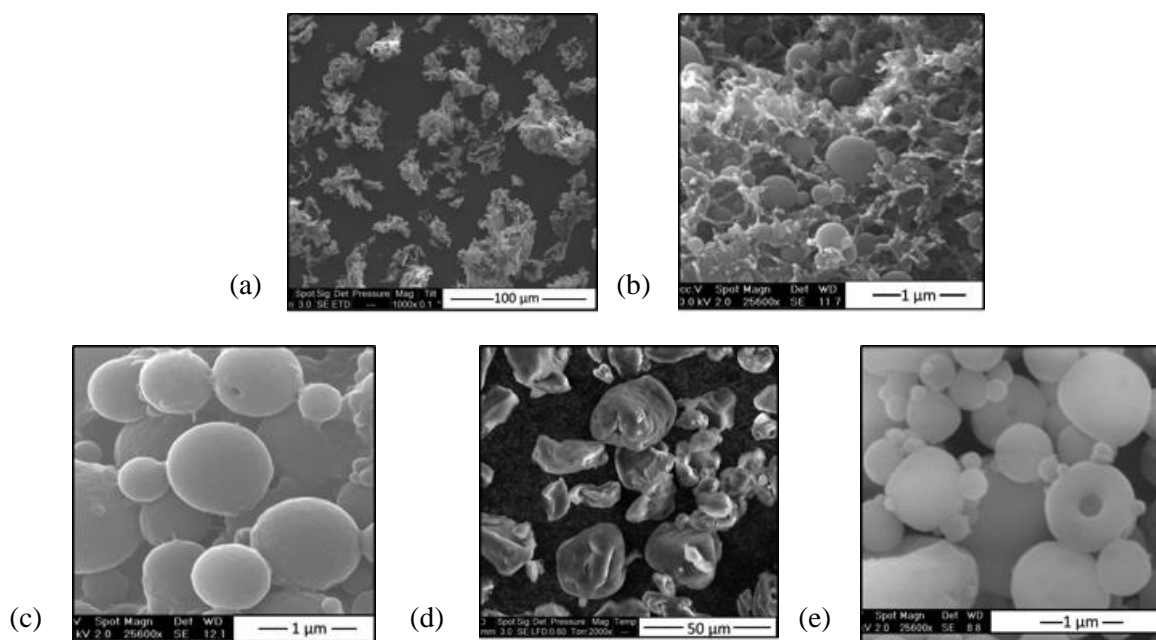


Figure 1. SEM photographs of chitosan particles generated by nebulization process (a), by SAS process for two potassium hydroxide concentrations (2% (b) and 1% (c)), commercial chitosan (d) and particles generated by SCASA process (e)

Using SCASA process, different chitosan suspensions were tested and at the end of the process, dried particles were directly collected in the filters. As shown in Figure 1(e), these particles are 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 particles 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 15% of the measured particles larger than $1 \mu\text{m}$) and microscaled particles (median diameter around $20 \mu\text{m}$) mainly composed of agglomerated nanoscaled particles.

3.2. PLA / chitosan films characterization

For pure PLA film and all composites films, the studied zone had a thickness between 30 and 90 μm . Independently of the type of chitosan particles, a yellowing of PLA films was observed when the particles were incorporated. During extrusion, in the same processing conditions, the presence of chitosan particles made the compound less viscous and the obtained films seemed to be more ductile than pure PLA films, for the same thickness. Further investigations will focused on the visco-elastic properties of the films.

The dispersion of chitosan particles inside the PLA films can be observed on the following optical microscope pictures (Figure 2). For all chitosan particle type, a good dispersion within the extruded PLA films was noticed. However, for the same weight ratio of chitosan and for the same magnification, different morphologies were observed depending on the particle type: distribution of particles in the “Com : PLA” films (Figure 2 (a)) was heterogeneous and showed large areas without chitosan particles. These areas were narrower for the “Nebu : PLA” films (Figure 2 (b)) and less noticeable for the “Nano : PLA” films (Figure 2 (c and c’ at higher magnification)). This can be explained by a larger specific area of chitosan nanoparticles compared to nebulized and commercial particles, which allowed a better dispersion in the PLA film.

Generally, no significant agglomeration was noticed regardless the type of chitosan particles. Commercial chitosan particles were easily identified. Nebulized particles were porous and have a flaky needle and random shape. Chitosan nanoparticles were well dispersed and only few large particles were observed, potentially due to remaining agglomerated nanoparticles and mainly to some impurities of residual materials from previous experiments. Several superimposed layers of these thin particles were observed within the film thickness, which made the observation harder.

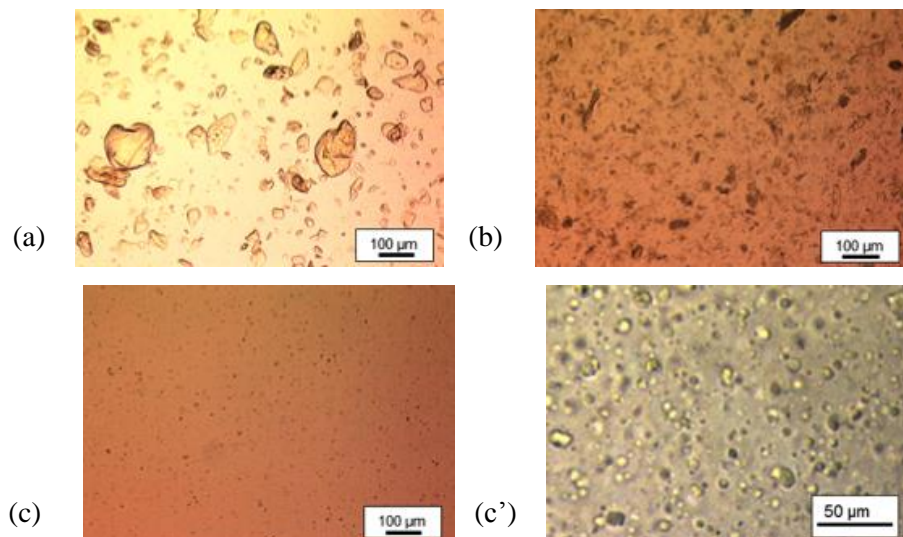


Figure 2. Photographs showing the dispersion of chitosan particles in PLA films: (a) Com : PLA, (b) Nebu : PLA, (c et c’) Nano : PLA

Figure 3 shows the first and the second DSC heating scans of the studied films. The first heating is representative of the effect of the process and the particle loads and types on the PLA crystalline structure, whereas in the second heating scan shows only the effect the

particle loads and types (the effect of the extrusion process was eliminated after the first melting). Comparing the two heating scans, no significant changes in the glass transition temperature T_g (60°C) and the melting temperatures were observed. However, the crystallization exothermal peaks were shifted to higher temperatures indicating that the cast extrusion process induced an earlier cold crystallization of PLA, possibly due to oriented mesophases that favour the crystallisation of the PLA chains.

On the second melting scan, “Pure PLA”, “Com : PLA” and “Nebu : PLA” films showed the same behaviour: a cold crystallization around 131°C, 129°C and 128°C respectively, and a melting around 154°C, 155°C and 154°C respectively. However, an obvious change was observed for the “Nano: PLA” film: a narrower crystallization peak at a lower temperature (118°C) indicating an earlier cold crystallization compared to the other films. These two phenomena suggest a nucleating effect of the chitosan nanoparticles. Moreover, two endothermic peaks around 152°C and 156°C were observed for both heating scans implying the presence of two defined crystalline structures.

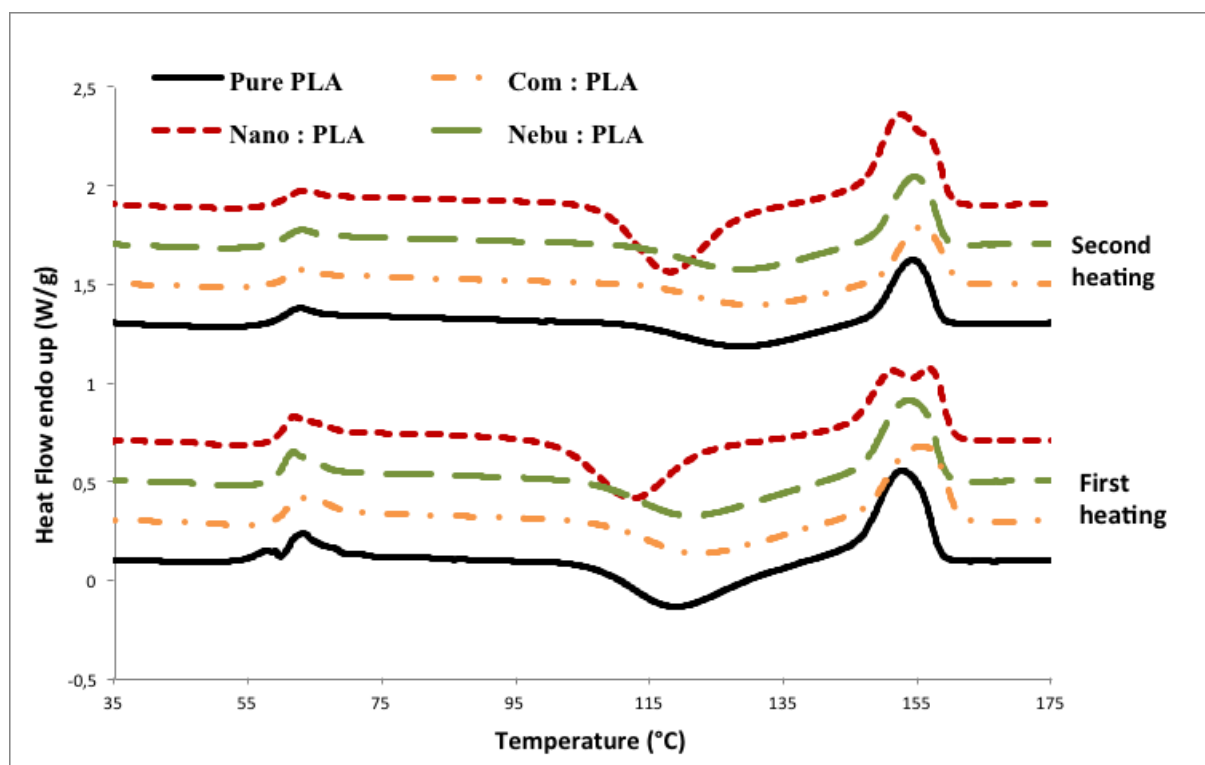


Figure 3. First heating and second heating DSC scans for “Pure PLA”, “Com : PLA”, “Nebu : PLA” and “Nano : PLA” films

4. Conclusion

Chitosan nanoparticles were generated using two supercritical CO₂ assisted processes: the supercritical antisolvent process led to average size particles of 378 ± 13 nm; however a better understanding and control of the physico-chemical phenomena especially in the alkaline recovery solution should allow a better stability of the particles in suspension. Dried particles with a median diameter of 400 nm were obtained by supercritical CO₂ assisted solubilisation and atomization process; it is simple, organic solvent-free and repeatable process,

nevertheless, an optimization of the operating parameters is still needed to obtain homogeneous sized particles. The generated particles, as well as the commercial chitosan, were incorporated in biobased PLA films (2.4% w/w) by cast extrusion. The morphology and properties of the resultant nanostructured composite films were studied: all particles were well dispersed in PLA films with no significant agglomeration; however chitosan nanoparticles, having a larger specific area than other particles, covered a greater area of the film. Differential scanning calorimetry revealed a nucleating effect of the chitosan nanoparticles. A further step will consist in studying the viscoelastic properties of the composites as well as the antimicrobial activities.

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