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Formation of chitin nano-fibers by supercritical antisolvent

José F. Louvier-Hernández and Gabriel Luna-Bárcenas*
CINVESTAV, Unidad Querétaro, Libramiento Norponiente No. 2000, Fracc. Real de Juriquilla Querétaro, Qro., 76230 México

Ranjit Thakur and Ram B. Gupta
Department of Chemical Engineering, Auburn University, Auburn, AL, 36849 USA
*Corresponding author. Phone: (442) 441-4908; Fax: -4938; email: gluna@qro.cinvestav.mx

Abstract

Chitin is emerging as a biopolymer of choice due to its potential uses in the numerous biomedical and pharmaceutical applications (e.g., wound dressing, tissue engineering, drug delivery, anti-allergic agent, etc.). Nanofibers can provide enhanced properties in many of these applications. Unfortunately, due to highly crystalline nature, chitin is not soluble in conventional solvents, and it is very difficult to convert it into the fine particulate or fibrous forms. In this work, chitin is dissolved in hexafluoroisopropanol solvent. This solution is then sprayed into supercritical carbon dioxide, which rapidly removes the solvent and precipitating chitin as nano-fibers. Based on scanning electron microscopy, precipitated chitin is a web of nano-fibers of about 84 nm in diameter. According to the Fourier-transform infrared spectroscopy, chitin molecular structure is preserved during the processing.

Introduction

Chitin, the second most abundant natural polymer after cellulose, is commonly found in the exoskeletons or cuticles of many invertebrates and in the cell walls of most fungi and some algae. It is usually obtained from the shells of shellfish, crab, lobster, or shrimp. Chitin, like cellulose, is a glucose-based polysaccharide, and differs from cellulose by having and acetamido residue in place of a hydroxyl group at the C-2 carbon[1]. Chitin is not soluble in the most conventional solvents; hence it has been termed as "intractable" which has been the main reason for lack of published studies on chitin[2]. There are only a few "exotic" solvents that can solubilize chitin, including dimethylacetamide with 5 w/v% LiCl[3], methanol saturated with calcium chloride dihydrate[4], hexafluoroisopropanol (HFIP), and hexafluoroacetone sesquihydrate. The latter solvents appear to provide solubilization without alteration of the chitin molecular structure, for example, preparation of chitin films and fibers using HFIP as solvent were reported by Capozza[5,6], where he reported that chitin/HFIP solution is transparent but viscous even at 1.5 wt.% concentration, and there is no indication of polymer degradation.

In SAS, solution (e.g., solute + solvent) is sprayed through a fine nozzle into supercritical fluid which acts as an antisolvent. Supercritical carbon dioxide is the most widely used antisolvent for SAS process. The process is operated at conditions in which the solvent and antisolvent are miscible, and solvent has more affinity for antisolvent than solute, forming a homogeneous phase. Due to the rapid extraction of the solvent from the solution, super-saturation occurs, causing the solute precipitation. After the solute is precipitated, it is further washed with the supercritical antisolvent to remove any residual solvent, and then the system is depressurized for product collection.

Experimental section

Hexafluoroisopropanol (HFIP) was purchased from SynQuest Labs (>99% purity, Lot # Q 88-106) and was filtered through a PTFE syringe filter (0.2 micron pore size) prior to use. Liquid carbon dioxide was purchased from BOC gases (SFC/SFE grade 5.5) and was used as received. Chitin from crab shells was purchased from Sigma (Practical grade, Batch # 033K1181) with 20 mesh size and a 96% degree of acetylation as reported by the manufacturer. Since the original chitin contains small amounts of proteins and mineral, it was used after purification, as reported by Clarke[14].

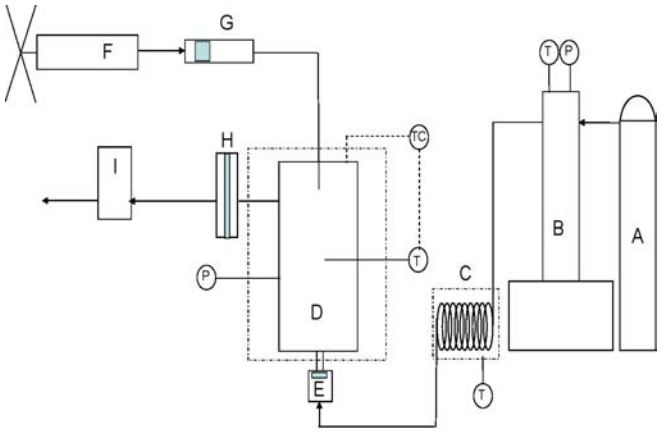

Figure 1. SAS apparatus showing (A) CO_2 tank, (B) ISCO syringe pump, (C) preheating coil, (D) precipitation vessel, (E) 0.5 micron stainless steel frit, (F) HIP hand pump, (G) injection device, (H) high pressure filter holder, and (I) backpressure regulator.

Chitin was mixed in HFIP to obtain 2.0 mg/ml concentration, and then stirred for 48 h. After this period most of the chitin was dissolved, except a small amount, which was filtered through a 0.2 micron PTFE syringe filter before using in SAS.

A schematic of the SAS apparatus used to produce the chitin fibers is shown in Figure 1, and the procedure is described elsewhere[7]. In these SAS experiments, temperature was set to 40 °C and pressure to 103.4 bar. Enough CO_2 flow was used to ensure a single phase (supercritical), and this was verified experimentally performing one experiment injecting 5.0 mL only of HFIP solvent (which is the maximum quantity of solution injected). The HFIP/CO_2 mixture forms a single phase.

Results and discussion

When the chitin/HFIP solution is injected into supercritical carbon dioxide, a fast precipitation of chitin in fiber form occurs. Most SAS processing of organic materials yields precipitate in the particulate form. However in the present case, due to strong intra-molecular h-bonding in chitin, fibers were obtained. The material obtained is cream in color (Figure 1Figure 2), and is extremely fluffy with an estimated bulk density of about 0.01 g cm^{-3}. When handling with tweezers, the fibers appears to be sticky.

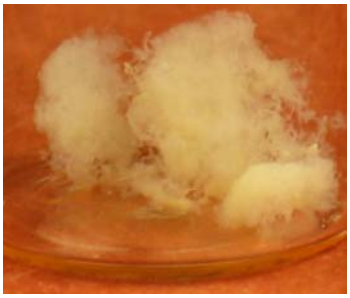

Figure 2. Chitin fibers obtain from SAS process

Figure 3d and Figure 3e are 20,000x magnifications which clearly show the presence of nano-fibers. The diameters of about two hundred fibers were manually measured. The average diameter was found to be 84 nm with standard deviation of 26 nm.

Figure 4 shows the spectra for original (purified) chitin (a) and chitin nano-fibers obtained from different runs of SAS process (b, c, and d). The spectra of the processed chitins are similar to that of the original chitin. Hence, the molecular structure is preserved after SAS processing. The infra-red spectrum of purified chitin is shown in Figure 4(a) is also similar to that reported by Gow and Gooday[15].

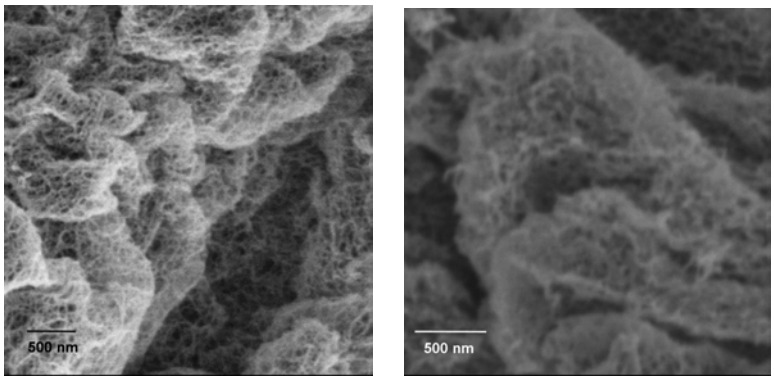

Figure 3. SEM micrographs of obtained chitin nano-fibers

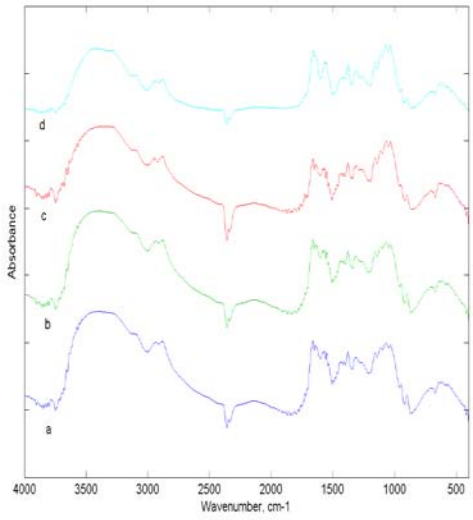

Figure 4. Infrared spectra of: (a) original chitin, and (b, c, d) SAS processed chitin nano-fibers

Figure 5 shows the diffractograms for original (purified) chitin (in red), which shows well resolved peaks at 9.8°, 13.14°, 19.9°, 23.78°, 26.78°, 35.29°, 39.8°, and a shoulder at 21.27°. The first six peaks and the shoulder have been reported by Jaworska et al[16], because they presented their figures in the 2θ angle range between 3° and 30°. The diffractogram for commercial purified chitin (Sigma) was obtained and it is in good agreement with that of purified chitin. After processing chitin, the crystalline structure seems to disappear in the chitin nano-fibers.

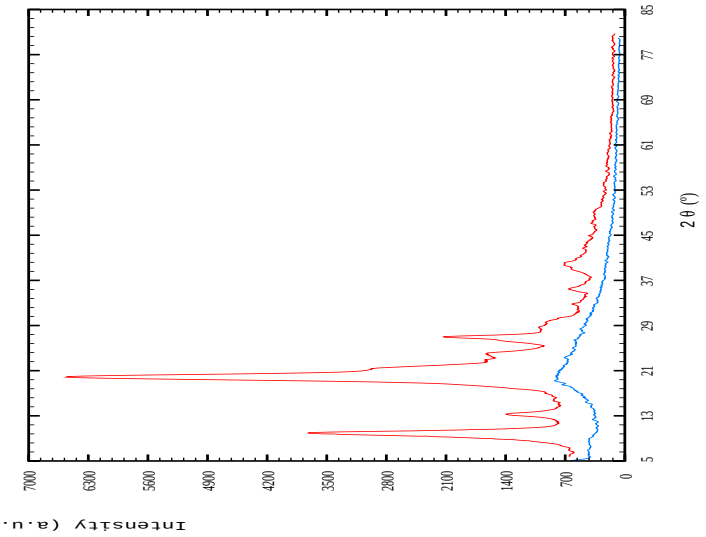

Figure 5. XRD paterns of: (red) original chitin, and (blue) SAS processed chitin nano-fibers

Conclusion

Nano-fibrous chitin can be successfully produced using supercritical antisolvent process. The obtained nano-fibrous chitin is very porous and sticky, and has very low bulk density. The average diameter of the nano-fibers is 84 nm. Infra-red analyses show that the molecular structure is not altered during the supercritical antisolvent processing. X-ray diffraction pattern of nano-fibers suggest an amorphous structure, which is in good agreement with the fact that faster precipitation occurs in the SAS process, leading to disordered structures.

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Financial support from NSF (project CTS-0219271) for the USA team and from NSF-CONACyT and CONCYTEQ (project 106/02) for the Mexican team is greatly appreciated. José F. Louvier-Hernández thanks COMEXUS for the Fulbright-García Robles scholarship.

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