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Bio-inspired production of chitosan/chitin films from liquid crystalline suspensions

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ABSTRACT

Inspired by chitin based hierarchical structures observed in arthropods exoskeleton, this work reports the capturing of chitin nanowhiskers' chiral nematic order into a chitosan matrix. For this purpose, highly crystalline chitin nanowhiskers (CTNW) with spindle-like morphology and average aspect ratio of 24.9 were produced by acid hydrolysis of chitin. CTNW were uniformly dispersed at different concentrations in aqueous suspensions. The suspensions liquid crystalline phase domain was determined by rheological measurements and polarized optical microscopy (POM). Chitosan (CS) was added to the CTNW isotropic, biphasic and anisotropic suspensions and the solvent was evaporated to allow films formation. The Films' morphologies as well as the mechanical properties were explored. A correlation between experimental results and a theoretical model, for layered matrix' structures with fibers acting as a reinforcement agent, was established. The results evidence the existence of two different layered structures, one formed by chitosan layers induced by the presence of chitin and another formed by chitin nanowhiskers layers. By playing on the ratio chitin/chitosan one layered structure or the other can be obtained allowing the tunning of materials' mechanical properties.

1. Introduction

Nature has always delighted us with its ability to evolve highly specialized biological systems based on straightforward bottom-up self-assembly processes (Aziz & Sherif, 2015; Seeman & Belcher, 2002). Applying only a few basic components, the smart organization of lipids, proteins, minerals and polymers across different length scales has conducted to astonishing structural and functional materials like bones in vertebrates, exoskeletons in arthropods, nacre in mollusks and cell walls in plants. The specific design of nanostructures and their assembly into hierarchical larger macrostructures allows the unique simultaneous combination of features like high strength, low weight, fracture toughness, and stimuli-responsive adaptability that are only virtually available in natural tissues and are far more developed than those currently achieved by man-designed materials (Egan, Sinko, LeDuc, & Keten, 2015; Nikolov et al., 2011). In particular, biomacromolecular structural materials such as collagen, cellulose and chitin are capable of forming complex topologies that can give rise to very interesting platforms gathering lightweight and stiffness with the ability to control the direction, color and polarization of light (Chung et al., 2011; Lu et al., 2013; Nguyen & MacLachlan, 2014; Shams, Nogi, Berglund, & Yano, 2012).

As the second most abundant polymer in the world, chitin is a well-known biomaterial that possesses unique properties like biodegradability, bioactivity, non-toxicity, antibacterial, antifungal and anti-inflammatory activity (Izumi et al., 2016; Qin et al., 2016; Robles, Salaberria, Herrera, Fernandes, & Labidi, 2016). This polysaccharide formed by poly-β-(1 → 4)-*N*-acetyl-D-glucosamine units is a fibrillar polymorphic semicrystalline polymer and the basic constituent of tissue nanostructures in different species (Gupta, 2010; Hamley, 2010). The aggregation of chitin's consecutive units leads to the formation of highly crystalline spindle-like fibrils often denominated chitin nanocrystals or chitin nanowhiskers (CTNW), that have aroused a growing interest in the scientific community due to their potential as reinforcements in nanocomposites (João, Silva, & Borges, 2015). Several methods like TEMPO-mediated oxidation (Fan, Saito, & Isogai, 2008a), mechanical grinding (Chen, Li, Hu, & Wang, 2014; Fan, Saito, & Isogai, 2008b), ultrasonication (Deng, Li, Yang, & Li, 2014)

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and high pressure homogenization (Salaberria, Fernandes, Diaz, & Labidi, 2015) have been successfully reported as capable of producing chitin with a diverse set of widths and lengths at the nanoscale and suggested their use as structural or functional reinforcements for multiple applications (João, Baptista, Ferreira, Silva, & Borges, 2016). Among all the methods, acid hydrolysis remains the approach capable of extract the narrow nano part of chitin – the nanowhisker – and consequently has been chosen in the present work (Sriupayo, Supaphol, Blackwell, & Rujiravanit, 2005; Tzoumaki, Karefyllakis, Moschakis, Biliaderis, & Scholten, 2015)

From the nano to the micro levels, chitin has the ability of producing long-ranged hierarchical structures within the organic matrix of crustaceans and insects' exoskeleton (Fig. 1). When observing shells' cuticle (Fig. 1) under polarized optical microscopy, the matrix reveals an ensemble of regular CTNW *laminae*, gradually rotating around the normal direction and forming a twisted plywood system (Bouligand structure). The long range packaging of *laminae* leads to the formation of solid three-dimensional patterns with strong anisotropy that constitute the source of structural color and mechanical integrity (Bouligand, 1972; Giraud-Guille, Belamie, & Mosser, 2004). These structures display textures similar to the domains formed in chiral nematic liquid crystals, which has contributed to the idea that biosynthesis of living tissues must involve, during one or more steps, liquid crystalline states of matter (Belamie, Mosser, Gobeaux, & Giraud-Guille, 2006).

Chitosan (CS), a chitin derivative obtained when sufficient acetyl groups are removed, can be found in some fungi cell walls and although less common in nature, it is intensively explored by men. It's a well-known natural polymer that preserves the majority of chitin's properties but in opposition does not share its hydrophobicity neither its lack of swelling capability. This polysaccharide has been largely explored mainly in bioscience fields covering a wide variety of forms like films, 3D structures, microspheres etc. CS ability to be dissolved in acidic aqueous media constitutes an enormous advantage for polymer processing and chemical modification (Desbrieres & Babak, 2010; Kim, 2013; Payne & Raghavan, 2007). Moreover, since CS is a chitin derivative, the chemical similarity between both favors their compatibility and this feature has been highlighted in several applications (Dutta, Dutta, & Tripathi, 2004; Rana & Fangueiro, 2016). The CS/CTNW combination was used in the production of films, hydrogels, fibers and scaffolds. In addition, CTNW have also been included in multiple nanocomposites as fillers reinforcing other polymer matrices like polycaprolactone, poly(vinyl alcohol), methacrylate and natural rubber (João et al., 2015). In all of those applications, the nanowhisker contribution in the improvement of composite mechanical properties has been reported. However, the reason why the reinforcement effects are lower than expected is still unknown. Small aspect ratios, lower chitin content or even whisker agglomeration has been pointed out as possible explanations (Mushi, Utsel, & Berglund, 2014). Furthermore, it is clear that polymer processing methods have not accomplished the production of materials with certain functional and structural capabilities that are only available in Nature's self-templated materials(Chung et al., 2011). The exploitation of chitin's ability to self-assemble in liquid crystalline structures is still limited and only few works regarding gelification of CTNW suspensions (Oh et al., 2016) and their biomineralization (Yamamoto, Nishimura, Saito, & Kato, 2010) were able to capture such organization. Polymer processing often leads to chiral nematic order disruption and that has been a major drawback in the production of structures with Bouligand-like architecture.

Therefore, in this work we propose a different bottom-up approach to maintain chitin's chiral nematic structure in a natural polymer matrix by producing chitosan/chitin films from liquid crystalline suspensions of chitin nanowhisker's. The analysis of films' mechanical properties revealed interesting features allowing to establish a tunable structure-properties relationship. Being able to mimic chitin's native structure and to capture its liquid crystalline order may open a new path for future application in the bioscience fields.

2. Experimental

2.1. Materials

Chitin from shrimp shells (C9213-1 kg; Lot# SLBB8542V; CAS: 1398-61-4) was purchased from Sigma Aldrich. Chitosan (CHITOPHARM S; Mw = 500 kDa; DD = 75%, CAS: 9012-76-4, Lot# UPBH8332PR) was purchased from Cognis. Acetic acid (glacial, 99.7%, Lot#266601, M = 60,06 g/mol, CAS: 131008-1212) and hydrochloric acid (37%, M = 36,46 g/mol, CAS:131020.1212) were supplied by Panreac. Water used was purified by a Millipore Elix Advantage 3 purification system.

2.2. Preparation of chitin nanowhiskers

CTNW were produced following the method proposed by Revol and Marchessault (1993). Briefly, 1 g of chitin was dissolved in 10 ml of HCl 3 M at 110 °C during 90 min. After cooling to room temperature, the solution was successively centrifuged (Heraeus, Multifuge X1R, Thermo Scientific) for periods of 15 min at 11,000 rpm, with the addition of Millipore water between centrifugations and elimination of supernatant, until pH = 2. At this point, the resulting supernatants were kept and dialyzed against Millipore water until neutral pH was achieved. Final CTNW suspensions were freeze-drying for 24 h (Vaco 2, Zirbus).

2.3. Preparation of chitin nanowhiskers suspensions

CTNW suspensions (CTNW content of 0.5, 0.62, 0.95, 1.27, 1.5, 1.9, 2.25, 2.55, 2.99, 3.7, 4.87 and 7.5% wt/wt) were produced from the dialysis retentates. The concentrations of the retentates were determined by freeze-drying a known volume of dialysed solution and measuring the mass of the freeze-dried product. CTNW suspensions were prepared either by centrifugation (higher concentration) or dilution with water (lower concentration). The suspensions were then sonicated at low intensity (20–30% of 750 W) until a translucent suspension was achieved (max. time 30 min), indicating that nanowhiskers were well dispersed.

2.4. Production of chitin nanowhiskers/chitosan films

To obtain the polymeric films, 2% (v/v) acetic acid solution was added dropwise to a 1.9% wt/wt CTNW suspension until pH = 2.8 was achieved. Following the same procedures of centrifugation/dilution, combined solutions were prepared containing 1% wt/wt chitosan and three different CTNW content (0.75%, 1.5% and 3.0% wt/wt). Afterwards, the solutions were placed in petri dishes and water was allowed to slowly evaporate in an oven at 37 °C for 72 h, until thin films were obtained and constant weight established. The films were then stored in a vacuum desiccator.

2.5. Characterization

Rheological characterization of the CTNW suspensions was performed using a stress-controlled rheometer Bohlin Gemini HR nano, with a cone-plate geometry (20 mm diameter and 2°). Temperature during the test was kept at 25 °C. The evolution of viscosity with shear rate was recorded at room temperature for a shear rate range of 0.01–1000 s^{-1}. All the measurements were obtained in steady state conditions.