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# Effect of soluble polysaccharides addition on rheological properties and microstructure of chitin nanocrystal aqueous dispersions

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ABSTRACT

Mixtures of chitin nanocrystal aqueous dispersions (at pH 3.0) with soluble polysaccharides of varying molecular features were examined rheologically and microscopically, under different conditions of biopolymer concentration, ionic strength, pH and temperature. The addition of non-adsorbing polysaccharides (guar gum, locust bean gum and xanthan) as well as oppositely charged (κ-carrageenan) to a chitin nanocrystal dispersion, resulted in a network formation and the gel strength increased with the chitin nanocrystal concentration. In contrast, the chitin nanocrystal – chitosan or – pullulan mixed dispersions did not show any network formation ($\tan\delta > 1$) at the concentration range examined. An increase in ionic strength and pH also resulted in an enhanced elasticity of the chitin nanocrystal–guar gum dispersions. Furthermore, an increase in the elastic modulus, which was irreversible upon cooling, was observed upon heating the chitin nanocrystal–polysaccharide mixed dispersions.



## 1. Introduction

Polysaccharide nanocrystals from various sources such as starch, cellulose and chitin have been receiving plenty of attention lately, due to their distinguished physical properties (Dufresne, 2011; Lin, Huang, & Dufresne, 2012). Moreover, chitin has attracted a lot of interest because of its plethora of biological properties. Chitin is a structural biopolymer found in shellfish, insects, and microorganisms and is the second most abundant polysaccharide found in nature. It can be hydrolyzed with hydrochloric acid to produce a dispersion of colloidal chitin nanocrystals that are positively charged due to the protonation of the amino groups present in the chitin molecules (Belamie, Davidson, & Giraud-Guille, 2004; Lin, Huang, & Dufresne, 2012; Marchessault, Morehead, & Walter, 1959; Revol & Marchessault, 1993; Zeng, He, Li, & Wang, 2012). Chitin nanocrystals (also called nanofibrils or nanowhiskers) have been used in various biomedical applications, such as wound medicaments and anti-inflammatory agents (Azuma et al., 2012; Muzzarelli, 2012; Muzzarelli & Muzzarelli, 2005; Muzzarelli et al., 2007) or as mechanically reinforcing biodegradable particles (Dufresne, 2011; Zeng et al., 2012).

Because of the rod-like shape of the chitin nanocrystals, these dispersions display liquid crystalline behavior above a critical particle concentration, as proposed by Onsager's theory for rigid rod-like particles (1949). At low chitin nanocrystal concentrations the dispersions are isotropic, with a random arrangement of rods, whereas at high concentrations the dispersions are anisotropic, with the chitin rods developing a birefringent nematic-like structure. Just beyond the critical concentration for anisotropic phase formation is a biphasic region in which the isotropic and anisotropic phases coexist (Revol & Marchessault, 1993). In addition to the isotropic–anisotropic transition, another phenomenon may take place, the anisotropic–nematic gel formation, which is usually caused with a further increase of particle concentration (Tzoumaki, Moschakis, & Biliaderis, 2010; Wierenga, Philipse, Lekkerkerker, & Boger, 1998). The physical origin of gelation of these dispersions is not fully understood, since it may involve different mechanisms depending on the type of interactions operating in such systems, either of repulsive or attractive nature (Buining, Philipse, & Lekkerkerker, 1994; van Bruggen & Lekkerkerker, 2002; Wierenga et al., 1998). Some of the most important variables that affect the rheological behavior of such dispersions are particle concentration as well as factors which influence the strength of inter-particle

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interactions; i.e. the nature of particles themselves (size and size distribution, shape and surface charge), the ionic strength and pH of the aqueous medium (ten Brinke, Bailey, Lekkerkerker, & Maitland, 2007).

Mixing the anisotropic particle dispersions with soluble polymers is another way to modulate the phase behavior and the mechanical properties of these systems (Aarts, Tuinier, & Lekkerkerker, 2002; Beck-Candanedo, Viet, & Gray, 2007; Buitenhuis, Donselaar, Buining, Stroobants, & Lekkerkerker, 1995; ten Brinke et al., 2007); the characteristics of such mixtures would mainly depend on differences in the physical properties and structure (size, shape, conformational flexibility, or charge) of the rod-like particles and the soluble polymer. For example, in the case of a non-adsorbing added polymer, it has been widely shown that if a structural property, such as shape or flexibility of the two components is different enough, a bulk demixing can occur in their mixed dispersions (Beck-Candanedo et al., 2007; Edgar & Gray, 2002; Flory, 1978). In this context, a random coil polymer will be excluded from an anisotropic phase consisting of rod-like particles. These thermodynamically unfavorable interactions arise mainly from excluded volume effects, governed by the physical volume occupied by one biopolymer molecule that is inaccessible to the other biopolymer molecules (Semenova, 2007). In general, depletion-type interactions occur from the imbalance in osmotic pressure that results when the polymer molecules are excluded from the area between two colloidal particles, where the inter-particle distances are smaller than the polymer effective diameter, resulting in an attractive force between the colloids (Adams, Digic, Keller, & Fraden, 1998; Asakura & Oosawa, 1954, 1958; Tuinier, Aarts, Wensink, & Lekkerkerker, 2003; Tuinier et al., 2008). There are studies showing that the addition of a flexible polymer in rod-like particle dispersions, like cellulose nanocrystals (Beck-Candanedo et al., 2007; Edgar & Gray, 2002) and boehmite rods (Buitenhuis et al., 1995), may lead to an isotropic–nematic transition in systems of lower anisotropic particle content. Moreover, in other studies it was found that the addition of a relatively flexible polymer induces aggregation and gelation (van Bruggen & Lekkerkerker, 2000), and increases the elastic modulus of colloidal dispersions (Fan & Advani, 2007). These changes in rheological properties have been attributed to formation of a percolated filler network in the polymeric matrix (Fan & Advani, 2007; van Bruggen & Lekkerkerker, 2000). On the other hand, the addition of an oppositely charged polymer in a rod-like particle dispersion, would be expected to result in associative phase separation (or else called complex coacervation) (de Kruif & Tuinier, 2005; Kruif, Weinbreck, & de Vries, 2004).

In an effort to modulate the mechanical properties of chitin nanocrystal aqueous dispersions, the impact of soluble polysaccharides with varying molecular characteristics, like molecular conformation, charge and size, on the rheological behavior and microstructure of mixtures containing chitin nanocrystals was examined. The selected soluble polysaccharides were guar gum, locust bean gum and xanthan gum, κ-carrageenan (a negatively charged polysaccharide), chitosan (a positively charged polysaccharide) and pullulan as a particularly flexible neutral biopolymer. Solutions of these polysaccharides were mixed with aqueous chitin nanocrystal dispersions and the resulting mixtures were studied with dynamic rheometry under different conditions of soluble polysaccharide-particle concentrations, ionic strength, pH and temperature. Also, complementary polarized optical micrographs have been captured in an attempt to relate the rheological behavior and stability of the composite dispersions to microstructural changes. Some of the results presented herein are those for guar gum, unless otherwise stated, since similar behavior was observed for some of the soluble polysaccharides tested in this study.

## 2. Materials and methods

### 2.1. Materials

Chitin from crab shells, hydrochloric acid (concentrated 37% v/v), sodium acetate, glacial acetic acid, potassium hydroxide, sodium chlorite and sodium chloride were purchased from Sigma Chemicals (St Louis, MO). Instantized guar gum (carbohydrates as dietary fiber > 94% d.b., protein ~4% d.b.) and locust bean gum (carbohydrates as dietary fiber > 92% d.b., protein ~5% d.b.) were obtained from Zumbro River Brand Inc. (Owatona, US), kappa carrageenan (Genugel®) and xanthan (Keltrol®) were obtained from CP Kelco (Denmark), pullulan (PI 20, purity > 93% d.b., Mw ~ $360 \times 10^3$; Lazaridou, Biliaderis, & Kontogiorgos, 2003) from Hyashibara Biochem. Laboratory Inc. (Okayama, Japan), and chitosan (Chitosan 500, degree of deacetylation 83%, Lot No. CTA080521) was purchased from Seikagaku Corporation (Tokyo, Japan). Double distilled water was used in all the experiments.

### 2.2. Chitin nanocrystals preparation

Aqueous stock dispersions of chitin nanocrystals were prepared by acid hydrolysis (3 M HCl, 95 °C, 90 min) of the original raw crude chitin material from crab shells (Sigma Chemicals). Detailed information on the isolation protocol is given elsewhere (Tzoumaki et al., 2010).

### 2.3. Sample preparation

The solid chitin content of the stock chitin nanocrystal dispersion was determined gravimetrically by drying aliquots of the sample at 50 °C until a constant weight was obtained; the total solids content of the stock dispersion was approximately 2.7% w/w (pH 3.0). Stock solutions of the soluble polysaccharides were also prepared at pH 3.0. The pH of the samples was adjusted at 3.0 by using HCl solutions. Appropriate quantities of the soluble polysaccharide solutions, and chitin nanocrystal dispersion were mixed in order to prepare dispersions of varying polymer composition. Additionally, the effect of small electrolytes was investigated by adding salt at different concentrations.

For all the experiments, the stock chitin nanocrystal dispersion was first placed in an ultrasound bath (Ultrasons-H, P Selecta, Spain) in order to disrupt any weakly formed aggregates (15 mL portions for 1 min) before the addition of any soluble polysaccharide.

### 2.4. Instrumental analyses

#### 2.4.1. State diagrams

The rheological behavior of the mixed dispersions was characterized by dynamic oscillatory measurements and samples with $G' > G''$ at 1 Hz were regarded as gels; measurements were taken after 5 min of sample loading in the rheometer, unless otherwise stated.

Guar gum and κ-carrageenan concentrations were measured at the upper phase when macroscopic phase separation occurred, by the phenol-sulfuric method for total carbohydrates (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using solutions of the respective polysaccharides as standards.

#### 2.4.2. Rheological measurements

Rheological measurements of the samples were performed by a rotational Physica MCR 300 rheometer (Physica Messtechnik GmbH, Stuttgart, Germany) using a double-gap geometry (internal and external gap 0.42 and 0.47 mm, respectively) in a controlled