Carbohydrate Polymers 95 (2013) 324–331

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

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

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ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 25 February 2013
Accepted 27 February 2013
Available online 6 March 2013

Keywords:
Chitin nanocrystals
Guar gum
Kappa carrageenan
Pullulan
Chitosan
Rod-like particles
Nematic gel
Rheological properties
Phase separation
Microstructure

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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http://dx.doi.org/10.1016/j.carbpol.2013.02.066

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 × 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 shear-stress mode. The temperature was regulated by a Paar Physica circulating bath and a controlled peltier system (TEZ 150P/MCR) with an accuracy of ±0.1 °C. To minimize dehydration, the samples were covered with a thin layer of silicone oil.

The linear viscoelastic region (LVR) was assessed at 1 Hz by amplitude sweep experiments; for all the chitin nanocrystal dispersions a constant deformation of $\gamma = 0.001$ was applied, which was within the linear viscoelastic region of all the samples. Small deformation oscillatory measurements for evaluation of the viscoelastic properties, G' (storage modulus), G'' (loss modulus), and $\tan\delta$ (G''/G'), were performed over the frequency range 0.01–100 Hz at 20 °C.

In order to explore the behavior of the chitin nanocrystal–guar gum mixed dispersions upon heating, samples were heated from 20 °C to 86 °C at a scan rate of 3 °C/min and then cooled back to 20 °C at the same scan rate. All the experiments mentioned above were carried out at a constant frequency of 1 Hz.

Flow curves were obtained at stresses ranging between 0.01 and 100 Pa.

2.5. Microscopy

Optical micrographs of the chitin nanocrystal–soluble polysaccharide dispersions were captured by an Olympus BX 51 polarizing optical microscope fitted with a digital camera (Olympus, DP 50). The specimens were prepared by placing a thin layer of the sample between a microscope slide and a coverslip.

3. Results and discussion

3.1. State diagrams

The regions characterized as “gel” and “liquid” on the state diagrams (Fig. 1) were defined by oscillatory rheometry measurements. For an ideal gel which behaves elastically, the G’ value is expected to be independent of frequency and $G' >> G''$. “Gels” herein are considered less restrictively, when the samples had $G' > G''$, at 1 Hz. The two different states detected in aqueous mixed dispersions of chitin nanocrystal–soluble polysaccharides were dependent on polymer composition. It was also observed that the chitin nanocrystal–κ-carrageenan mixed dispersions (Fig. 1b) presented a gel-like behavior at lower polymer concentrations compared to the other mixtures (Fig. 1a, c and d). On the other hand, the chitin nanocrystal–pullulan (Fig. 1c) dispersions presented gel-like behavior only at high concentrations of chitin nanocrystal, 2.0% w/w, a value close to which a chitin nanocrystal dispersion by itself forms a weak gel (2.4% w/w) (Tzoumaki et al., 2010). The latter mixed system showed similar behavior even at pullulan concentrations as high as 5% w/w (inset Fig. 1c). The chitin nanocrystal–chitosan dispersions also presented a liquid-like behavior over the entire chitosan concentration range examined (Fig. 1d).

In Fig. 2a, the respective regions of gel and liquid on the state diagram for chitin nanocrystal–guar gum mixed dispersions is given for a greater range of concentrations, together with the macroscopic phase separation line (dotted line) after 48 h at ambient conditions. It was noted that below the phase separation threshold, the chitin nanocrystal–guar gum mixtures yielded two distinct phases after 48 h: one translucent and one opaque as seen in the images in Fig. 2a. The opaque phase most probably contains most of the chitin nanocrystals of the mixture, since it has been observed that the chitin nanocrystal aqueous dispersions exhibit an opaque appearance, even at low concentrations (Tzoumaki et al., 2010). Therefore, it could be assumed that the translucent upper phase contains mainly guar gum; however, it was not clear to what extent the guar gum preferably partitions between in the upper vs. the lower chitin nanocrystal phase. For that reason, the guar gum concentration was quantified (data not shown) in the upper-transparent phase, and it was found that there is a small (within the experimental error limit) preference for selective partition of the guar gum to the upper translucent phase. Moreover, the samples with higher chitin nanocrystal and guar gum content did not show any macroscopic phase separation, probably because of the increased viscosity and the formation of a network that hinders completion of the phase separation events between the two polymeric components. It was also noticed (Fig. 2a) that the macroscopic phase separation boundary was a little higher than the liquid-gel transition line and this probably occurred because the samples below the phase separation line were not strong enough gels to inhibit the phase separation.

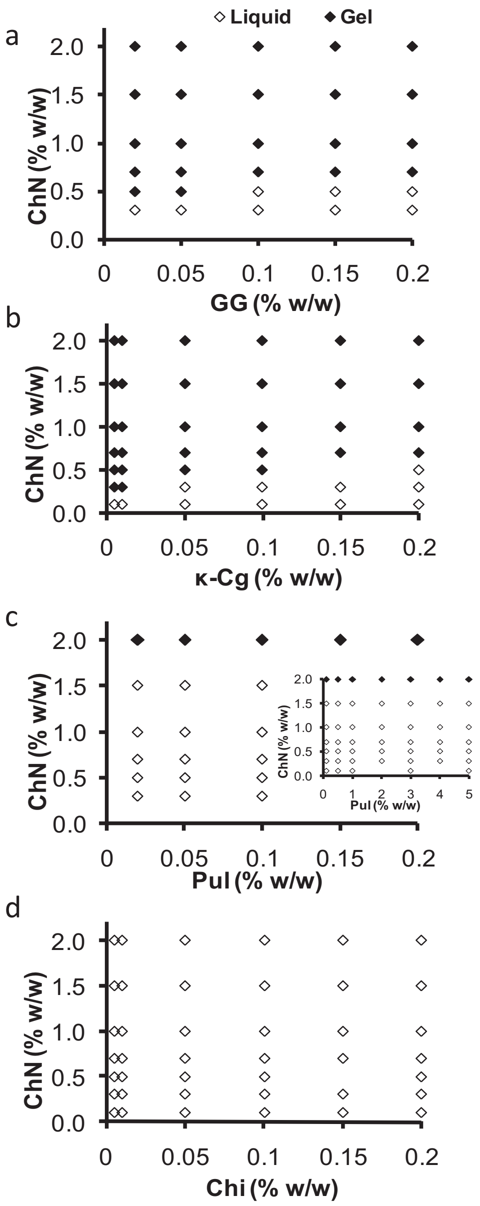

Fig. 1. State diagrams for mixed chitin nanocrystal (ChN)–soluble polysaccharides aqueous dispersions (pH 3.0): the gel state was defined rheologically when the sample exhibited the condition $G' > G''$ at 1 Hz. (a) ChN-GG (Guar gum); (b) ChN-κCg (κ-carrageenan); (c) ChN–Pul (Pullulan); (d) ChN–Chi (Chitosan).

In Fig. 2b, the state diagram of the mixed chitin nanocrystal–guar gum dispersions containing NaCl (100 mM) is also given and it is

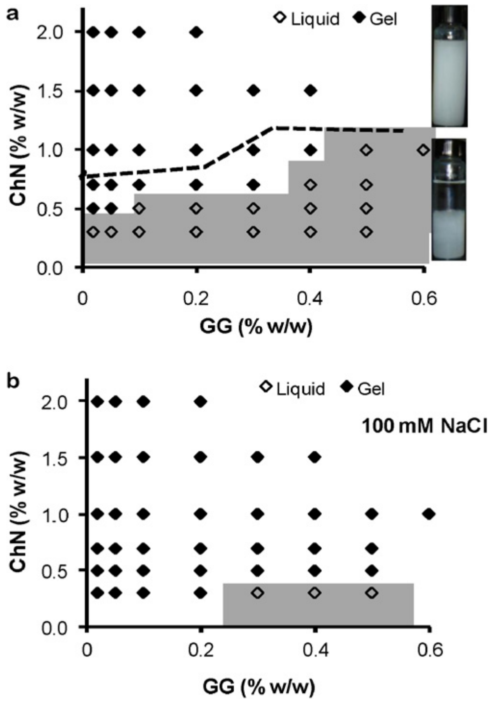

Fig. 2. (a) State diagram for mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions (pH 3.0): the gel state was defined rheologically when the sample exhibited the condition $G' > G''$ at 1 Hz. The dotted line shows the "limit" for macroscopic phase separation after 48 h storage at ambient conditions; (b) state diagram for mixed ChN–GG aqueous dispersions containing NaCl 100 mM.

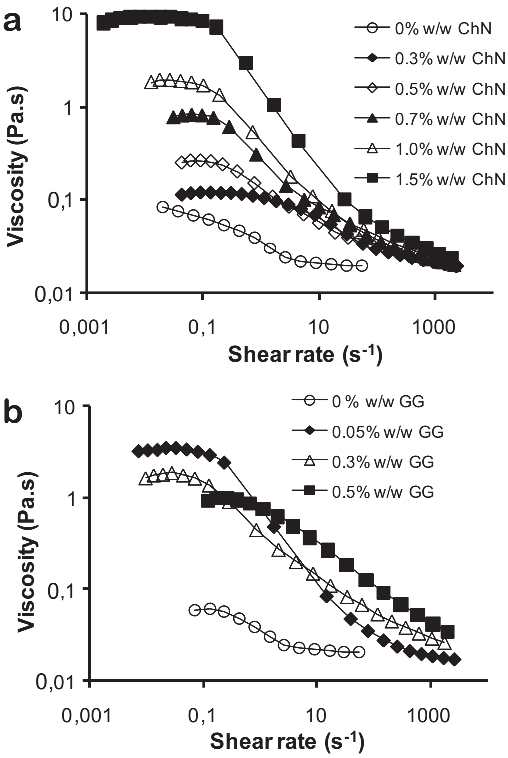

Fig. 3. Flow curves of mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions (20 °C) (pH 3.0): (a) effect of chitin nanocrystal (ChN) concentration by keeping constant the guar gum (GG) concentration at 0.2% (w/w); (b) effect of guar gum (GG) concentration by keeping constant the chitin nanocrystals (ChN) concentration at 1.0% (w/w).

noticed that the samples presented a gel-like behavior at lower concentrations of chitin nanocrystals compared to the respective samples with no added salt. Salt addition leads to increased attractive interactions among chitin nanocrystals due to the electrostatic screening of the charged alike chitin rods, therefore the network structure becomes stronger, and no macroscopic phase separation is observed (no phase separation line). Previous studies involving addition of salt in chitin nanocrystal dispersions (Belamie, Davidson, & Giraud-Guille, 2004; Tzoumaki et al., 2010) indeed showed that their viscoelastic properties were enhanced with addition of electrolyte. Moreover, Lowys, Desbrieres, and Rinaudo (2001) have found that for cellulosic microfibril dispersions, the higher the sodium chloride concentration, the stronger the gel-like behavior. In contrast, no actual network strengthening of the mixed chitin nanocrystal – whey protein dispersions was observed, even when the salt concentration was as high as 300 mM (Tzoumaki, Moschakis, & Biliaderis, 2011).

3.2. Rheological and microstructural properties

3.2.1. Effect of concentration

The flow curves of the chitin nanocrystal–guar gum mixed dispersions (Fig. 3) show that the samples exhibit shear thinning behavior with high viscosity at low shear stresses, implying the formation of a transient network. The increase in chitin nanocrystal concentration from 0.3 to 1.5% w/w (Fig. 3a), while keeping guar gum concentration constant (0.2% w/w), leads to a significant increase in viscosity at low shear rates (zero shear viscosity) by two orders of magnitude, indicating the predominant role of the chitin nanocrystals in structure formation. On the other hand, the addition of a relatively low concentration of guar gum (0.05% w/w) in chitin nanocrystal dispersion (1.0%) (Fig. 3b) resulted in an almost two orders of magnitude increase of the low shear rate viscosity. However, a further increase of the guar gum concentration from 0.05 to 0.5% w/w, led to a small decrease in low shear viscosity of the mixed dispersions. It is worth pointing out that these high values of zero shear viscosity obtained for the mixed dispersions could not be achieved by chitin nanocrystal or guar gum alone, at the respective levels of concentration for the two components, as it can be seen in Fig. 3.

For the 1.5% w/w chitin nanocrystal–0.2% w/w guar gum mixture, the G' was always higher than G'' over the entire frequency range explored, and a weak frequency dependence was observed (Fig. 4a), typical of weak gel behavior (Tzoumaki, Moschakis, & Biliaderis, 2011; Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011). On the other hand, at a lower concentration (0.3% w/w) of chitin nanocrystals (0.2% w/w guar gum), the G'' was higher than G' (Fig. 4a) and a typical cross-over for liquid to solid-like behavior at high frequencies (viscoelastic liquid) was observed. At this point it is worth noting that a chitin nanocrystal dispersion at pH 3.0, without any soluble biopolymer added, behaves as a weak gel only when the concentration is slightly above 2.4% w/w (Tzoumaki et al., 2010). Moreover, with increasing chitin nanocrystal concentration from 0.3 to 2.0% w/w, while keeping the guar gum concentration constant at 0.2% w/w, the G' is raised by approximately two orders of magnitude, while the tanδ decreased (frequency 1 Hz), as shown in Fig. 4b. On the contrary, even though the addition of guar gum in chitin nanocrystal dispersions leads to remarkable increases in gel strength, in values that could never be achieved by each polymer alone, a further increase of the guar gum concentration, from 0.02 to 0.4% w/w, while keeping the chitin nanocrystal concentration constant at 1.0% w/w, seems to decrease the G', while the tanδ values increase, as shown in Fig. 4c.

The addition of the negatively charged κ-carrageenan, led to a remarkable increase in network strength of the chitin nanocrystal dispersion, especially at low concentrations of the added

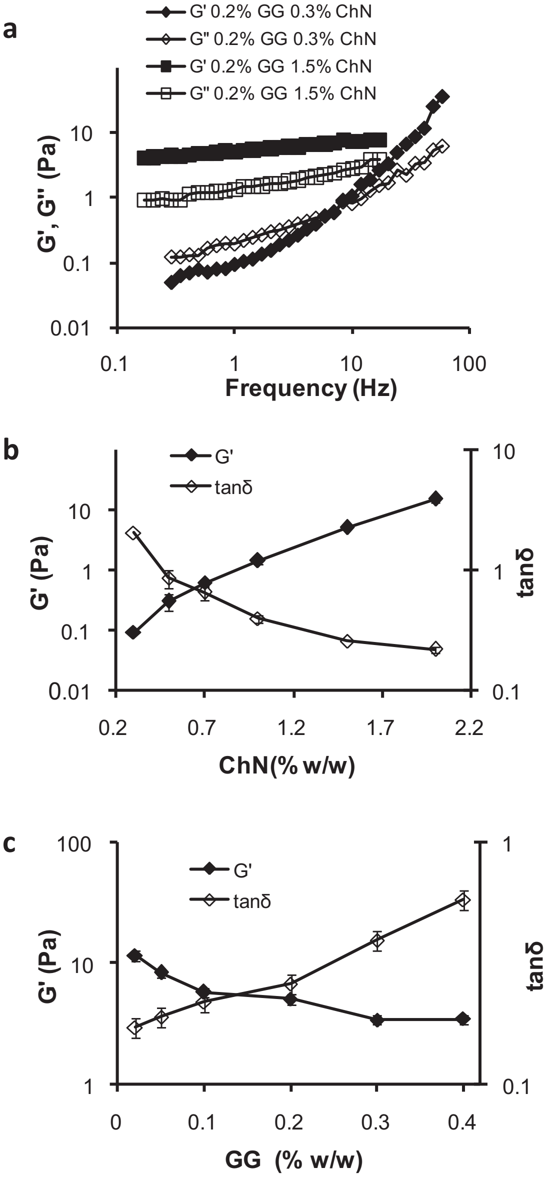

Fig. 4. (a) Mechanical spectra (20 °C, $\gamma = 0.001$) of mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions; (b) chitin nanocrystal (ChN) concentration dependence of elastic modulus (G') and tangent value (tanδ) of mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions (concentration of GG 0.2% w/w); (c) guar gum (GG) concentration dependence of elastic modulus (G') and tangent value (tanδ) of mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions (concentration of ChN 1.0% w/w). Conditions for rheological measurements: 20 °C, frequency 1 Hz, half bars represent one S.D.; all dispersions had pH 3.0.

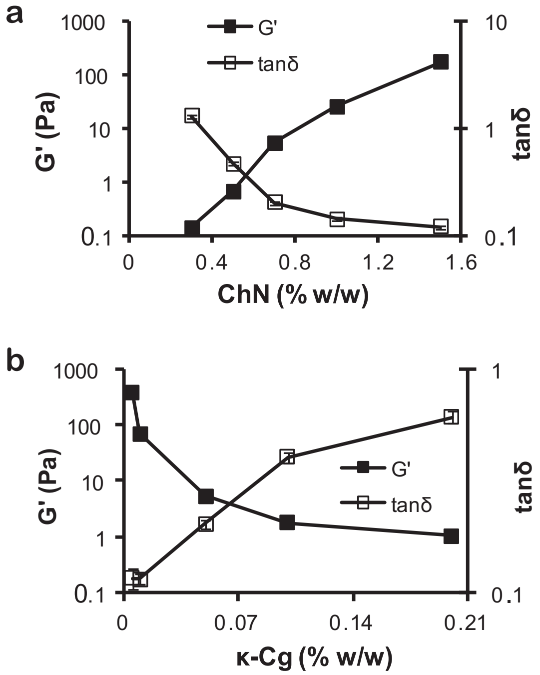

Fig. 5. (a) Chitin nanocrystal (ChN) concentration dependence of elastic modulus (G') and tangent value (tanδ) of mixed chitin nanocrystal-κ-carrageenan (ChN–κCg) aqueous dispersions (constant κCg 0.05% w/w); (b) κ-carrageenan (κCg) concentration dependence of elastic modulus (G') and tangent value (tanδ) of mixed chitin nanocrystal-κ-carrageenan (ChN–κCg) aqueous dispersions (constant ChN 0.7% w/w). Conditions for rheological measurements: 20 °C, frequency 1 Hz, half bars represent one S.D.; all dispersions had pH 3.0.

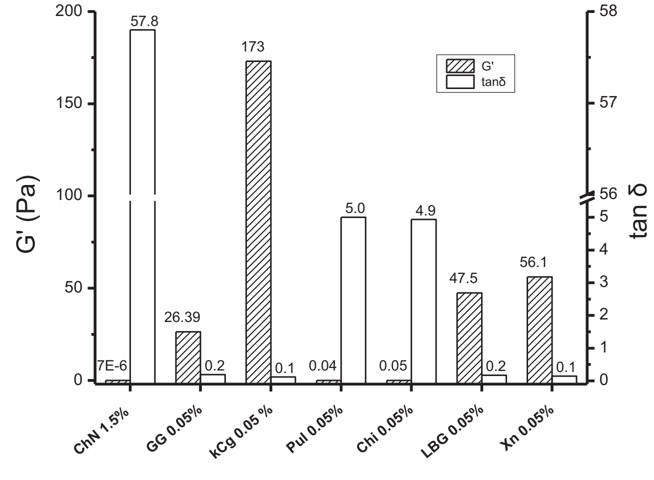

Fig. 6. G' and tanδ (at 1 Hz and 20 °C) of chitin nanocrystal-soluble polysaccharides mixed dispersions (pH 3.0); all samples contained 1.5% w/w chitin nanocrystal (ChN) and 0.05% w/w soluble polysaccharide.

soluble biopolymer. Fig. 5a shows that with increasing chitin nanocrystal concentration from 0.3 to 1.5% w/w, while keeping the κ-carrageenan concentration constant at 0.05% w/w, the G' is raised by approximately three orders of magnitude, while the tanδ decreased (frequency 1 Hz). Instead, with increasing the κ-carrageenan concentration, from 0.02 to 0.2% w/w, while keeping the chitin nanocrystal concentration constant at 0.7% w/w, there is a considerable decrease of the G' by approximately three orders of magnitude as well as an increase in the tanδ values (Fig. 5b); the pronounced reduction in the elastic modulus G' with increasing concentration of κ-carrageenan could be attributed to the increase in viscosity of the continuous phase, by the excess of κ-carrageenan not adsorbed on chitin nanocrystals, in levels that the movement of chitin nanocrystal is hindered, so that they cannot easily align and form nematic network structures. The chitin nanocrystal–κ-carrageenan mixed dispersions also presented macroscopic phase separation (data not shown) after 48 h at ambient conditions, at low biopolymer concentrations, similar with the chitin nanocrystal–guar gum mixtures.

In Fig. 6 the respective G' and tanδ values for all chitin nanocrystal (1.5% w/w)–soluble polysaccharide dispersions of similar concentrations are given, in an attempt to compare their rheological properties and relate them with their molecular characteristics. It is observed that the chitin nanocrystal–guar gum, chitin nanocrystal–κ-carrageenan, chitin nanocrystal–locust bean gum and chitin nanocrystal–xanthan mixed dispersions exhibited

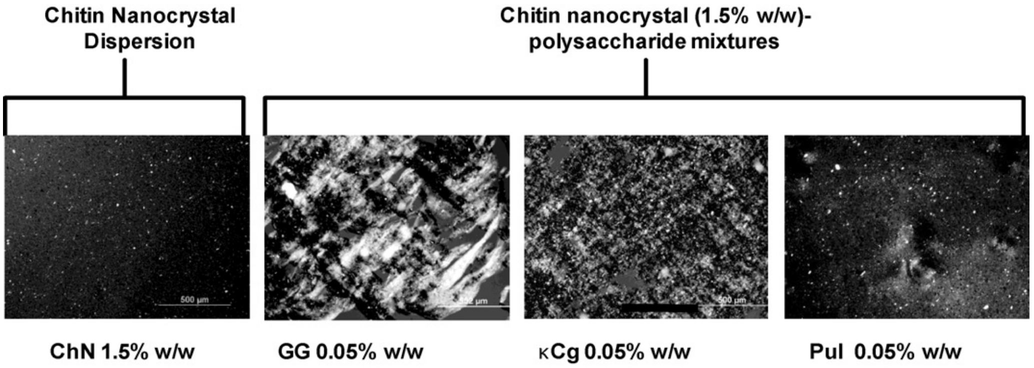

Fig. 7. Typical polarized optical micrographs of a chitin nanocrystal (ChN) aqueous dispersion (1.5% w/w) and of mixed chitin nanocrystal (ChN)–soluble polysaccharides dispersions; all samples had pH 3.0.

a gel-like behavior, with their tanδ values being below 1; it was also interesting to note that the chitin nanocrystal–κ-carrageenan mixed dispersion exhibited a G' value distinctly higher than the other three samples. In comparison, it is important here to mention that the chitin nanocrystal dispersion at 1.5% w/w on its own, exhibited a liquid-like behavior, with the tanδ value being 57.8, and the G' considerably lower than the above mentioned mixed samples (about 7×10^{-6} Pa). In contrast to the above mixed dispersion systems, it was noted that the chitin nanocrystal–pullulan and chitin nanocrystal–chitosan dispersions showed a liquid-like behavior, with tanδ values above 1.

Some representative images of the chitin nanocrystal–soluble polysaccharide mixtures are given in Fig. 7, further pointing to the diversity in microstructural features of the aqueous chitin nanocrystal-polysaccharide dispersions. The 1.5% w/w chitin nanocrystal dispersion alone exhibited a dark non-birefringent image, while the chitin nanocrystal–guar gum mixture presented a birefringent-nematic structure, typical for nematic gels (Tzoumaki et al., 2010). The chitin nanocrystal–κ-carrageenan sample also displayed birefringent structures, while the chitin nanocrystal–pullulan dispersion did not show any evidence for birefringence.

From all the above, gel formation in the mixed chitin nanocrystal–guar gum, –locust bean gum, –κ-carrageenan and –xanthan dispersions at pH 3.0 is evident. In the case of guar gum locust bean gum and xanthan (pK ≈ 3), which can be considered as non-adsorbing biopolymers, it could be speculated that the network formation is mostly due to excluded volume effects (Dogic, Purdy, Grelet, Adams, & Fraden, 2004; Semenova, 2007; Tolstoguzov, 2003). The rods, like chitin nanocrystals, that exhibit significantly large excluded volume due to their anisotropic shape, and coils, like guar gum, are mutually excluded, as predicted theoretically by Flory (1978), who stated that with the addition of coils replacing the rods in the isotropic phase, the rod concentration in the nematic phase increases. This phenomenon has been shown to occur for several mixed colloidal systems (Adams et al., 1998; Beck-Candanedo et al., 2007; Dogic et al., 2004; Ramzi, Borgstrom, & Piculell, 1999). In the present system, increased chitin nanocrystal concentration means gel strengthening and sometimes the enhancement is such that may hinder the macroscopic phase separation; nonetheless, it may occur locally, at a microscopic scale (Tzoumaki et al., 2010, 2011). On the other hand, the reduction in the elastic modulus value with increasing guar gum concentration is probably caused by the increase in viscosity of the continuous phase to the extent that movement of chitin nanocrystal is hindered, so that they cannot form nematic network structures. For gel network formation in the chitin nanocrystal–κ-carrageenan mixed dispersions, an additional reason could be electrostatic interactions among κ-carrageenan chains, which are negatively charged and the chitin nanocrystals which are positively charged at pH 3.0, thus forming complexes that may give rise to weak gel-like behavior. Measurements of the distribution of κ-carrageenan in phase separated samples of chitin nanocrystal–κ-carrageenan mixtures, showed a preferential partition of the soluble polysaccharide in the lower opaque chitin nanocrystal-containing phase, suggesting possible adsorption of κ-carrageenan on chitin nanocrystal.

Instead, pullulan or chitosan, even though they could be considered as non-adsorbing biopolymers, with the former being non-charged and the latter being positively charged, similar to chitin nanocrystal, did not alter the rheological properties of the mixed dispersions, in the concentration range examined. In the case of pullulan, it could be speculated that it does not have a large excluded volume due to its particularly flexible structure (Lazaridou et al., 2003) and thus, it does not cause pronounced phase separation of the chitin nanocrystal dispersions. In contrast, the guar gum and locust bean gum exhibit relatively more rigid structures and large excluded volume effect (Laguna, Tarazona, & Saiz, 2003) and this seems to bring about more distinct phase separation in the chitin nanocrystal dispersions. In the case of chitin nanocrystal–chitosan mixed system, since both chitin nanocrystal and chitosan originate from chitin, they would not exhibit unfavorable interaction between similar segments and as such they may not cause mutual exclusion and phase separation between the two components.

3.2.2. *Effect of temperature*

The influence of temperature on the behavior of chitin nanocrystal–guar gum mixed dispersions (chitin nanocrystal 1.0% w/w and guar gum 0.2% w/w) was studied by applying a heating–cooling cycle on samples containing different chitin nanocrystal concentrations (the guar gum concentration was kept constant at 0.2% w/w) from 20 to 74 °C. As shown in Fig. 8, the heating–cooling process resulted in an irreversible increase in the elastic modulus of the mixed dispersions. The increase in elastic modulus upon heating was greater with increasing chitin nanocrystal concentration (Fig. 8), and this increase most likely reflects strengthening of the chitin nanocrystal network itself. Indeed, in our previous works (Tzoumaki et al., 2010, 2011) it was shown that heating causes an increase in storage modulus of aqueous chitin nanocrystal dispersions, which is irreversible upon cooling, and the enhancement in G' values depends on the upper temperature. It was also found, that the chitin nanocrystal dispersions are not stable over time even at 20 °C, and as the particles tend to re-orientate to give a stronger network structure, heating seems to accelerate this process; i.e. the kinetic energy of the particles is increased and thus they become more mobile to rearrange themselves. Moreover, with increasing temperature, the kinetic energy of the counterions increases and the diffuse-double layer thickness is reduced.

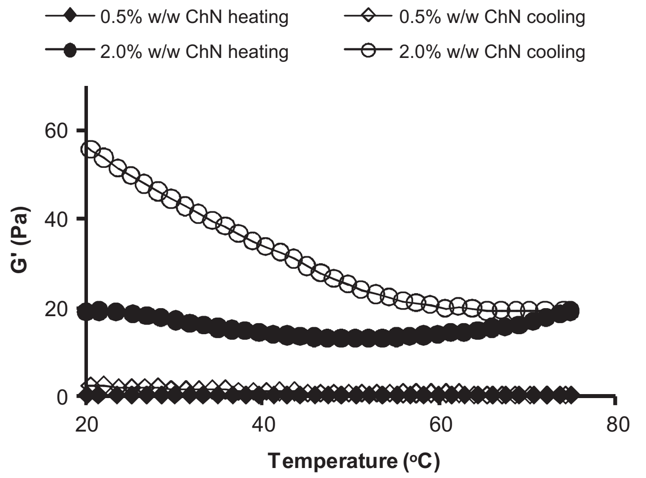

Fig. 8. Effect of heating at 74 °C and cooling to 20 °C on the elastic modulus (G') of mixed chitin nanocrystal-guar gum (ChN–GG) aqueous dispersions (ChN 0.5% or 2.0%, GG 0.2% w/w). Heating and cooling rate at 3 °C/min, frequency of 1 Hz, and pH 3.0.

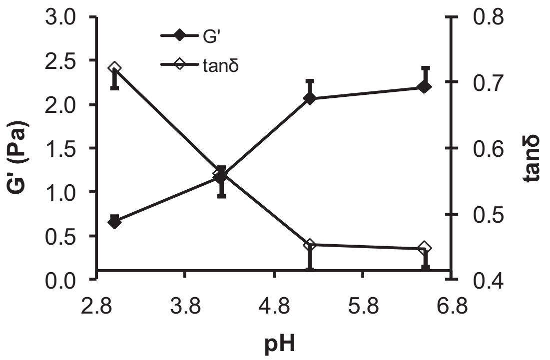

Fig. 9. Variation of G' and tanδ of mixed chitin nanocrystal (ChN) (1.0% w/w)–guar gum (GG) (0.2% w/w) aqueous dispersions as a function of pH (half bars represent S.D., $n = 3$).

As a result, the interparticle distances decrease and the associative forces between the nanocrystals become stronger (Tzoumaki et al., 2010). It has been argued that the attractive interactions which are dominant in the system of chitin nanocrystal aqueous dispersions are most probably of van der Waals type, since the changes observed upon heating are irreversible (Tzoumaki et al., 2010).

3.2.3. *Effect of pH*

Similar responses to those obtained with NaCl addition were also noted for the effect of pH. As it can be seen in Fig. 9, with increasing pH the chitin nanocrystal–guar gum mixed dispersions exhibited a stronger gel-like behavior; i.e. the G' value increases and the tanδ decreases. Since the isoelectric point of chitin nanocrystal dispersions is around 6.3 (Li, Revol, & Marchessault, 1997), therefore as the pH reaches lower values, e.g. pH 3.0, the chitin nanocrystal amino groups should be totally protonated. The strong electrostatic repulsive forces at this pH level would reduce the tendency for network formation. In contrast, when the dispersion approaches a pH value of 6.3, the repulsive electrostatic forces are minimized and the chitin nanocrystal particles could then easily aggregate and form a gel, as observed in the case of chitin nanocrystal dispersions without any addition of soluble biopolymers (Tzoumaki et al., 2010).

4. Conclusions

The addition of soluble polysaccharides, like guar gum, locust bean gum, κ-carrageenan and xanthan, in a chitin nanocrystal dispersion, which initially did not behave elastically, resulted in a network formation and the gel strength was amplified with increasing chitin nanocrystal concentration. The gel structure formation can be attributed to phase separation phenomena, in the case of non-adsorbing guar gum and locust bean gum and xanthan, possibly caused by excluded volume effects; i.e. when the biopolymer is excluded from the interspace between the chitin nanocrystal colloidal particles, the imbalance in osmotic pressure finally leads to attractive forces between the colloidal particles, as described early by Asakura and Oosawa (1958). In the case of the oppositely charged κ-carrageenan, the gel strengthening could be attributed to electrostatic interactions. On the other hand, addition of a relatively more flexible biopolymer coil such as pullulan did not bring about any pronounced effect in rheological properties of the chitin nanocrystal dispersion. Moreover, the soluble polysaccharide, chitosan did not seem to cause extensive phase separation in the chitin nanocrystal dispersions, at the concentrations employed, possibly due to its similar with chitin nanocrystal chemical structure. Among all hydrocolloids tested, the negatively charged polysaccharide, κ-carrageenan, seemed to exert the strongest impact in network formation in the mixed dispersions. However, the interactions governing the phase behavior of the above mixed biopolymer systems are not clear, since gel formation could result from a complex interplay of different forces. Heating of the chitin nanocrystal–soluble polysaccharide mixed systems led to further enhancement in the storage modulus, which was irreversible upon cooling. Similarly, increasing the ionic strength or the pH brought about strengthening of the composite network structure, possibly due to electrostatic screening of the chitin nanocrystal particles. All the above lead to the conclusion that the addition of soluble polysaccharides in chitin nanocrystal dispersions is an effective way to modulate the rheological properties of the chitin nanocrystal–soluble polysaccharide mixed dispersions and produce novel structures with interesting textural properties in food systems.

Acknowledgements

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek National Funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II, “Investing in knowledge society through the European Social Fund”.

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