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Hydrophobized cellulose nanocrystals enhance xanthan and locust bean gum network properties in gels and emulsions

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Abstract

Locust bean/xanthan gum (LBG/XG) synergistic networks have previously been well studied, with evidence that junction zones between the two polymers result in hydrophobic domains. Here we report on the effect of both hydrophilic and hydrophobic cellulose nanocrystals (CNCs) on the rheological properties of the individual gums, the gum networks, and emulsion gels consisting of the gum network and corn oil. We also take advantage of differences in the autofluorescent spectra for each of the components to map their distribution within the gel and emulsion gel systems. Whilst both types of CNC confer thermal stability to the systems, hydrophilic CNCs induce minor changes in rheological properties of synergistic gels and prove to be detrimental to the stability of the emulsion gels. In contrast, hydrophobic CNCs associate with the LBG/XG network, affecting the rheological response. Their inclusion in the emulsion gel system results in smaller, more homogeneously distributed oil droplets with a resultant increase in the storage modulus by an order of magnitude compared to the CNC-free and hydrophilic CNC systems. We conclude that hydrophobic CNCs play a critical role in stabilising LBG/XG network gels and emulsions.

Key words: Locust bean gum, xanthan gum, cellulose nanocrystal, hydrophobicity, rheology, fluorescent spectroscopy

Abbreviations: CNCs, cellulose nanocrystals; LBG, locust bean gum; XG, xanthan gum; MCLSS, multi-channel confocal laser scanning spectroscopy

1. Introduction

Water-soluble polymers are essential for numerous applications in a wide range of industrial sectors such as food, cosmetics and personal care, agriculture, medicine, etc. The applications of these polymers are based on their ability to modify the rheological properties of water-based systems, induce gelation, stabilize complex mixtures, emulsify water immiscible fluids, and many other functional properties. Carbohydrate polymers have enormous potential in such applications not only because of their functional properties but also due to renewability of their sources, low cost, and sustainability. They are materials of choice in many food, pharmaceutical and cosmetic products, and their potential applications continue to grow.

Carbohydrate polymers are very versatile, which enables extensive options in the selection of the materials to match specific functional properties. Their properties can be further altered by combining different polysaccharides or mixing them with other hydrocolloids such as proteins (Norton & Frith, 2001; Tolstoguzov, 2006). Among such mixed systems, mixtures of carbohydrate polymers showing a non-additive enhancement of properties are of special interest. Mixtures of xanthan gum (XG), a microbial exopolysaccharide, with various galactomannans, such as locust bean gum (LBG), are amongst the most studied examples that exhibit synergistic interactions (Acar & Kurt, 2020; Casas & García-Ochoa, 1999; Copetti, Grassi, Lapasin, & Priel, 1997; Goycoolea, Richardson, Morris, & Gidley, 1995; Grisel, Aguni, Renou, & Malhiac, 2015; Jo, Bak, & Yoo, 2018). A combination of XG, which forms weak gels, and non-gelling galactomannans results in a significant increase in viscosity at low polymer concentrations and the formation of strong thermo-reversible gels at moderate polymer concentrations. Whilst the exact mechanism of the synergistic effect in these systems is still debated, it is commonly accepted that the synergy is driven by heterotypic intermolecular interactions. Somewhat counterintuitively for these highly hydrophilic polymers, there is evidence that hydrophobic effects contribute to these interactions and network properties. For example, the retention of a hydrophobic probe (ethyl decanoate) in the liquid phase of the LBG/XG system was higher than expected from the retentions of individual gum solutions. The enhancement of the hydrophobe retention was attributed to an increase in local hydrophobicity due to interactions of XG side chains with smooth (unsubstituted) segments of LBG (Grisel et al., 2015). Additionally, NMR studies demonstrated motional restrictions of the XG pyruvate group in the presence of LBG (Takemasa & Nishinari, 2016), which confirms involvement of this hydrophobic moiety at the terminal end of XG side chains in the intermolecular interactions between XG and LBG. This implies that the structural and rheological properties of LBG/XG mixtures can be further

modified with moderately hydrophobic additives. Thus, ternary systems of LBG, XG and small amounts of hydrophobized cellulose derivatives (methyl cellulose (Rinaudo & Moroni, 2009), hydroxypropyl methyl cellulose (Jo & Yoo, 2018)) form gels with enhanced viscosity and strength. However, studies on the impact of hydrophobic effects on the functional properties of synergistic LBG/XG gels are limited.

Polymer/(nano)particle interactions are also widely used for changing the structural and rheological properties of polymer solutions and gels (Chen et al., 2018; Schexnailder & Schmidt, 2009). The impact of silica nanoparticles on the rheology of LBG, or XG, led to the conclusion that hydrogen bonding results in the adsorption of these biopolymers to silica (Kennedy, Kent, & Brown, 2015). However, silica nanoparticles have a low aspect ratio. As a result, the effect of silica nanoparticles on the solution properties was only noticeable at high filler contents. 1 wt.% of silica induced a two-fold increase in the zero-shear viscosity of the LBG solution. However, the system transformed to a gel with 10 wt.% of silica nanoparticles. For the gelled LBG/XG mixture, the rheological properties did not change upon addition of 1 wt.% silica, whilst 10 wt.% caused significant enhancement in the gel viscoelasticity. Interestingly, despite this enhancement, the authors assumed that, in these particle-mediated gel networks, silica nanoparticles might mitigate the synergy between XG and LBG. Gelation of LBG solutions was also achieved at a significantly lower nanoparticle concentration (3 wt.%) in the case of cellulose nanocrystals (CNCs), which are high aspect ratio rod-type nanoparticles, confirming that LBG also adsorbs onto CNCs (Hu, Cranston, Ng, & Pelton, 2014).

CNCs, and other cellulose nanomaterials, offer a good nanoparticle alternative for designing hybrid soft materials based on particle-mediated gel networks using components from renewable, sustainable sources. Mild hydrophobization of CNCs results in amphiphilic particles of good colloidal stability in aqueous media (Nigmatullin et al., 2020). This hydrophobic modification enables assembly with other hydrophobes, or amphiphiles (Pourmoazzen et al., 2020) including polysaccharides resulting in structural, rheological and functional changes (Nigmatullin et al., 2019; Nigmatullin et al., 2018). CNCs have also attracted interest in emulsion stabilization due to their ability to assemble at the oil-water interface. The synergistic effect in emulsion stabilization was observed when CNCs were combined with soluble amphiphilic cellulose derivatives, such as hydroxyethyl cellulose (HEC) or methyl cellulose (MC) (Hu, Patten, Pelton, & Cranston, 2015). HEC and MC are adsorbing polymers towards CNCs, and both CNCs and soluble polymers were observed to colocalize at the oil-water interface. This decreased the oil droplet size and improved emulsion stability compared to emulsions stabilized

by CNCs, or the polymer, alone. When polymer solutions forming the emulsion continuous phase reach suitable concentrations, the oil droplets become entrapped within a gel matrix. Such soft materials are called emulsion gels or emulsion-filled gels (Farjami & Madadlou, 2019). Synergistic LBG/XG mixtures form gels at low concentrations. However, galactomannans and XG are not efficient emulsifying agents. Therefore, they are used in combinations with other emulsifiers, such as proteins and surfactants, for the formation of emulsion gels (Owens, Griffin, Khouryieh, & Williams, 2018; Patel, Rodriguez, Lesaffer, & Dewettinck, 2014; Yang et al., 2019).

Herein we hypothesize that hydrophobic effects can drive formation of a ternary particle-mediated network consisting of a synergistic LBG/XG mixture and hydrophobized CNCs. Given the strong possibility of efficient emulsion stabilisation by hydrophobized CNCs, we further hypothesize that such hybrid ternary gels would enable the formation of emulsion filled gels. To prove these hypotheses we use hydrophobic CNCs prepared by surface modification via octylamine coupling (Nigmatullin et al., 2020). The effect of hydrophilic and hydrophobic CNCs on the rheological and structural properties of solutions and gels was studied for solutions of single polysaccharide and synergistic LBG/XG gels at different polymer and CNC concentrations. Ternary hybrid gels were used for the formation of emulsion gels, with corn oil as the dispersed phase. Morphological and rheological properties of the emulsion gels were studied at two different polymer concentrations and oil phase contents and compared with emulsion gels formed by synergistic LBG/XG without CNCs.

2. Materials and methods

2.1. Materials

Cellulose nanocrystals in sodium form were supplied as an 11.3 wt.% aqueous slurry by Maine University Process Development Centre (USA, Orono, ME). Hydrophobization of the CNC surface was conducted via coupling of octylamine with aldehyde groups of oxidised CNCs according to a previously described procedure (Nigmatullin et al., 2019). Detailed characterisation of both parent and hydrophobized CNCs has been conducted previously (Nigmatullin et al., 2020). These hydrophilic and hydrophobized CNCs were used as never-dried materials with concentrations of 11.3 and 5.4 wt.% respectively. Both types of CNCs were gel-like materials at these concentrations. XG from *Xanthomonas campestris* and LBG from *Ceratonia siliqua* seeds (M_w approximately 310 kD) were purchased from Sigma–Aldrich

(Loughborough, UK). Prior preparation of an LBG stock solution was purified by preparation of 0.5 wt.% solutions in deionised water (80 °C, 30 min). This solution was centrifuged (10000 rpm, 15 min) and the clear supernatant was lyophilised. Similar treatment of XG did not result in the formation of a precipitate after centrifugation. Therefore, XG was used without further purification. Corn oil was purchased from a local grocery shop.

2.2. *Solution and sample preparations*

A 1:1 mass ratio LBG/XG mixture was used as most publications involving characterization of this binary system reported the strongest synergistic effect at this ratio in both dilute (Copetti et al., 1997) and concentrated solutions (Jo et al., 2018). All formulations were prepared using individual 1.4 wt.% stock solutions of LBG and XG. Both stock solutions were prepared by dissolving the required amounts of LBG and XG in deionised water (Millipore, 80 °C, 40 min). To produce the LBG/XG and hybrid gels the required equal amounts of gum stock solutions were mixed with the required amounts of CNC gels and deionised water (total mass 6 g, magnetic stirring, 80 °C, 30 min). For emulsion gels, the required amount of XG stock solution, which had a lower viscosity than the LBG stock solution, was diluted with the required amount of deionised water and, in the case of hybrid systems, mixed with the required amounts of CNC gels. This mixture was homogenised (Branson sonifier, 3 mm tip, 10 % power, 70 °C, 30 s) before the required amount of corn oil was added and emulsified (10 % power, 1 min, 70 °C). Finally, the required amount of LBG stock solution was added to the emulsion. The final formulations were quickly mixed with a spatula and sonicated (10 % power, 70 °C, 1 min). Gels and emulsion gels, after cooling, were stored overnight at room temperature and characterised after 24 h. Sample codes for the emulsion gels and their corresponding compositions are summarised in Table 1. All formulations were prepared in triplicate.

2.3. *Rheological measurements*

A Discovery HR-1 rotational rheometer (TA Instrument) with a Peltier plate as a temperature controller was used for the rheological measurements with a cone and plate configuration (stainless-steel cone geometry, diameter: 40 mm, angle: 4°). All samples were loaded at 70 °C. Immediately after the loading, a temperature ramp was conducted by cooling to 25 °C (cooling

rate: 5 °C min⁻¹, strain: 1.5 %, angular frequency: 6.28 rad s⁻¹). After reaching 25 °C, an isothermal oscillatory step was performed (30 min, strain: 1.5 %, angular frequency: 6.28 rad s⁻¹). This step was required to ensure that material properties were stabilised. In all materials, the storage and loss modulus did not change after 15 minutes. This was followed by frequency sweeps conducted in strain-controlled mode (strain: 1.5 %, angular frequency range: 0.4 to 100 rad s⁻¹). Finally, steady-state shear viscosity was measured (shear rate range: 0.01 to 100 s⁻¹). During the rheological experiments, the measuring system was covered with a humidity chamber to minimize water evaporation. **One rheological measurement was conducted for each sample in triplicate and results were reported as average values.**

Table 1. Emulsion gel sample codes and corresponding composition

Sample code	LBG/XG conc. [wt.%]	CNC type	CNC conc. [wt.%]	Corn oil conc. [wt.%]
0.4X20	0.4	None	0.0	20
0.4X40	0.4	None	0.0	40
0.6X20	0.6	None	0.0	20
0.4CNC20	0.4	Hydrophilic	1.0	20
0.6CNC20	0.6	Hydrophilic	1.0	20
0.4C ₈ CNC20	0.4	Hydrophobic	1.0	20
0.4C ₈ CNC40	0.4	Hydrophobic	1.0	40
0.6C ₈ CNC20	0.6	Hydrophobic	1.0	20
0.6C ₈ CNC40	0.6	Hydrophobic	1.0	40

2.4. Multi-channel confocal laser scanning spectroscopy (MCLSS)

Spectral images were acquired using a Zeiss LSM 880 confocal microscope (405 nm diode laser, 5.0 % power, Plan-Apochromat 10×/0.45 M27 objective, MBS-405 filter, 32 channels: $\lambda = 411$ -695 nm). 3D z-stacks were flattened to 2D images using the orthogonal projection tool in ZEN 2.6 (blue edition) (Carl Zeiss AG, Oberkochen, Germany) (Figure S1). Spectral analysis was performed using the unmixing tool. Three images were acquired for each condition.

3. Results and Discussion

3.1. Influence of CNCs on the rheology of single gum solutions.

The industrial importance of XG and LBG has meant that the rheology of these polysaccharides in aqueous solutions have been studied extensively (Sittikijyothin, Torres, & Gonçalves, 2005;

Song, Kim, & Chang, 2006). LBG is considered as a semi-flexible polymer attaining a coiled conformation in solution. As a result, LBG solutions exhibit rheological properties that are typical for entangled polymer solutions. XG in solution, on the other hand, is mainly modelled as semi-rigid rods with a tendency to self-associate. Due to such structural attributes, XG aqueous solutions behave as weak gels. The typical rheological behaviour of 0.5 wt.% LBG solutions (Figure 1a) is characterised by a strong frequency dependence of the shear elastic modulus, G' , ($G' \sim \omega^{1.7}$) and a **weaker** frequency dependence of the shear loss modulus, G'' , ($G'' \sim \omega^{0.90}$). These dependences approximate the terminal responses for disordered random coils in aqueous solutions where $G' \sim \omega^2$ and $G'' \sim \omega$. It is noted that G'' is larger than G' at angular frequencies below 70 rad s^{-1} . Thus, LBG solutions behave as viscoelastic fluids at low frequencies and as solid-like materials at frequencies higher than 70 rad s^{-1} . In contrast, the XG solution responds to an oscillatory deformation as a solid like material with G' dominant over G'' even at low frequencies (Figure 1b). At the same time, both moduli **are frequency dependent**: $G' \sim \omega^{0.2}$ and $G'' \sim \omega^{0.3}$. These indicate that XG formed a weak gel at 1.0 wt.%.

Figure 1. Comparison of mechanical spectra of (a) 0.5 wt.% LBG solution (black diamonds) and (b) 1.0 wt.% XG solution (black squares) with additional solutions containing 1.0 wt.% of hydrophilic (blue triangles) or hydrophobized (red inverted triangles) CNCs. G' : filled symbols, G'' : open symbols. Temperature: 25°C , strain amplitude: 1.5%. (Note: Error bars are smaller than the symbols).

Both hydrophilic and hydrophobized CNCs induce notable changes in the viscoelastic properties of LBG solutions (Figure 1a). Mixtures do not exhibit a crossover point ($G' = G''$) with G' being larger than G'' over the entire angular frequency range (0.4 to 100 rad s^{-1}). Thus, the addition of CNCs to the LBG solution transformed it into a gel-like material. This is more pronounced for the hydrophilic CNCs, which individually do not form a gel at 1 wt.%, compared to the hydrophobized CNCs, which do (Figure S2). The G' of the two gels containing LBG at 6.34 rad

s⁻¹ were equivalent to an 8 wt.% hydrophilic CNC gel and a 1.5 wt.% hydrophobized CNC gel respectively (Nigmatullin et al., 2019). Previously Hu, Cranston, Ng, & Pelton (2014) demonstrated that LBG adheres to the surface of CNCs. This implies that the CNCs are involved in the formation of a hybrid network with the dissolved LBG. Although only slight differences were observed in G' and G'' values for the LBG solution containing the same quantities of hydrophilic and hydrophobized CNCs, these materials responded differently to oscillatory shear deformation. At low frequencies (up to 15 rad s⁻¹) the LBG/octyl-CNC gel is characterised by a lower dependence on frequency ($G' \sim \omega^{0.1}$) compared to hydrogels containing hydrophilic CNCs ($G' \sim \omega^{0.2}$). This implies a more rigid gel structure in the presence of hydrophobized octyl-CNCs. However, in an atypical manner, the G' of the LBG/octyl-CNC gel progressively decreased with an increase in the angular frequency above 30 rad s⁻¹ and became lower than that of LBG gels with hydrophilic CNCs. It appears that the gel structure involving hydrophobized CNCs is more susceptible to higher shear rates, which are realised at higher frequencies. This observation was also confirmed in steady shear experiments (Figure S3). The Newtonian plateau viscosity of the LBG/octyl-CNC gel was higher than that of the hydrophilic CNC gel. However, the octyl-CNC modified gel viscosity in the shear-thinning region became lower than that of the hydrophilic CNC gel. We theorise that hydrophobization of the CNCs decreases their interaction with LBG compared to the hydrophilic CNCs, resulting in the observed differences.

In contrast to the LBG solution, the presence of both types of CNCs at 1 wt.% in the XG solution had minimal effect on the rheological properties (Figure 1b). Hydrophilic CNCs slightly increased G' whilst the addition of hydrophobized CNCs resulted in a slight decrease of the gel's elastic response. This occurred despite XG containing hydrophobic acetyl and pyruvate moieties, which could potentially be involved in association with the hydrophobic domains of octyl-CNCs. The minor changes in rheology of XG with the addition of CNCs suggested that interactions between XG and both CNCs were poor. This could be due to electrostatic repulsion between the anionic XG and negative surface charge of the CNCs (Nigmatullin et al., 2020). For the octyl-CNCs, their self-association due to the hydrophobic effect might also limit their inclusion in a continuous hybrid network.

The effect of CNCs on the rheology of single gum solutions observed in our study is in agreement with the results for the same polysaccharides with silica nanoparticles (Kennedy et al., 2015). 1 wt.% of silica nanoparticles did not have effect on rheology of XG solutions while inducing an increase in viscosity and viscoelastic moduli of LBG solutions. However, changes in the rheological properties of LBG are much more pronounced in CNC mixtures compared to those

with silica particles. At the same nanoparticle content, the viscosity at the Newtonian plateau increased almost 600 times in the presence of CNCs (Figure S3) while only a two-fold increase was observed when LBG was mixed with silica particles (Kennedy et al., 2015). Even 10 wt.% of silica nanoparticles did not have such an impact on the LBG rheological properties as 1 wt.% of CNCs.

3.2. CNCs in synergistic LBG/XG gels.

The LBG/XG polymer network is one of the most extensively studied binary mixtures with known synergistic effects (Casas & García-Ochoa, 1999; Copetti et al., 1997; Goycoolea et al., 1995). It is well-established that LBX/XG aqueous solutions form firm thermo-reversible gels while, as discussed in the previous section, single gums form weak gels, or viscous solutions. In this study, solutions with equal content of each polysaccharide formed self-supporting invertible gels at a total polymer concentration as low as 0.2 wt.% (Figure S4). The thermal response of such hydrogels at two polymer concentrations is demonstrated in Figures 2a and 2b. LBG/XG solutions exhibit two distinctive regions: at lower temperatures, the elastic response dominates over viscous flow ($G' > G''$), while at higher temperatures G'' is higher than G' . This is typical for gel-sol transitions. The temperature at which the LBG/XG gel 'melts' ($G' = G''$) is dependent on the total polymer concentration and shifts to higher temperatures as the concentration increases (45 °C at 0.2 wt.%; 50°C at 0.5 wt.%). Formation of strong gels in LBG/XG was demonstrated by frequency sweeps (Figure S5), which confirmed weak dependence of G' on the angular frequency. The power law index ($G' \sim \omega^n$) was below 0.06 for all samples and dependencies of G' and G'' on frequency were almost parallel. Although the exact mechanism of the synergistic effect in LBG/XG network remains a subject of debate, the prevailing view is that the strong gels are due to the formation of cooperative (heterotypic) junction zones (Goycoolea et al., 1995; Grisel et al., 2015). It is hypothesised that smooth (unsubstituted) segments of the LBG macromolecules interact with the side chains of XG molecules. There is specific evidence that the pyruvate group at the terminal end of the xanthan side chain is involved in this interaction (Takemasa & Nishinari, 2016).

Figure 2. (a) and (b) Temperature dependence of viscoelastic moduli. Angular frequency 6.28 rad s^{-1} , strain amplitude 1.5 %. G' closed symbols, G'' open symbols. (c) and (d) Comparison between the steady shear viscosity (closed symbols, left and bottom axes) and the complex viscosity (open symbols, right and top axes). Temperature 25°C . Pure LBG/XG gels (black circles); gels plus hydrophilic CNCs (blue triangles); gels plus hydrophobized CNCs (red inverted triangles). Total gum concentration: 0.2 wt.% (a) and (c); 0.5 wt.% (b) and (d). CNC concentration 1 wt.%. Curves in (c) and (d) are the averages of three measurements, error bars are smaller than symbols.

The introduction of both hydrophilic and hydrophobized CNCs into the LBG/XG synergistic gel led to significant changes of the viscoelastic properties, with a greater increase in the elastic modulus observed for hydrophobized CNCs. For hydrogels with a total gum concentration 0.2 wt.% (Figure 2a), G' increased five and twelve times in the presence of hydrophilic and hydrophobized CNCs respectively. This trend was still observed at a higher total gum concentration of 0.5 wt.% (Figure 2b), although the relative increase was reduced to 2.2 and 6.7 times respectively. Interestingly, unlike the native LBG/XG gels, the hybrid hydrogels continued to behave as solid-like materials ($G' > G''$) at higher temperatures.

Newtonian flow regions at low shear rates (Figure 2c,d) observed similar increases. Zero-shear viscosities increased for the hybrid hydrogels, more than doubling in the presence of

hydrophobized CNCs compared with hydrophilic CNCs. It has previously been observed that a volatile hydrophobic probe is better retained by the LBG/XG matrix than the individual gums. Grisel et al. (2015) suggested that this occurred due to an increase in local hydrophobicity because of the interaction between LBG and XG. This hypothesis suggests that the formation of stronger hydrogels with hydrophobized CNCs is due to an integration of the hydrophobized CNCs into the LBG/XG network driven by hydrophobic effects. MCLSS was used to confirm this. Intensity maps of the LBG/XG spectrum and corresponding CNC spectrum reveal that the hydrophilic CNCs (Figure 3a,b) are uniformly present across the observed sample volume, whilst the gum distribution is heterogeneous at the investigated concentration. In contrast, the hydrophobized CNCs (Figure 3c,d) preferentially interact with the hydrophobic domains present in the LBG/XG network, resulting in their distribution matching that of the gum network.

It is also apparent that the self-association of hydrophobized CNCs also contributes to the robustness of the hybrid networks. The addition of 0.5 wt.% octyl-CNC did not increase the viscosity over that of the unmodified gel (Figure S7), unlike the 1.0 wt.% addition. Rather, the viscosity in the shear thinning region decreased compared to that of the pure LBG/XG gel. It is probable that, at higher polymer/CNC ratios, the hydrophobized CNCs are coated with the soluble polysaccharides and are well separated from each other, decreasing their interaction and, thus, the viscosity.

Figure 3. Representative MCLSS images of (a, b) LBG/XG gum with hydrophilic CNCs and (c, d) LBG/XG gum with hydrophobized CNCs. (a, c) are frontal (X,Y) projections of the gels whilst (b, d) are transverse (X,Z) projections of the same samples (Figure S1). Sample maps are intensity maps of the corresponding spectrum. Spectra available in SI (Figure S6). All scale bars represent 200 μm .

A notable feature of the flow curves (Figure 2c,d) for the LBG/XG gel and hybrid hydrogel with hydrophilic CNCs was a modest shear thickening that preceded the shear thinning region. **Figure S8 represents the flow curves in a semi-logarithmic plot for better visualisation of pattern differences. The shear thickening at small shear rates** has been observed in various polymer solutions including polysaccharide solutions (Kjøniksen, Hiorth, & Nyström, 2005). It is probable that re-arrangement of gum molecules within a specific range of relatively small shear rates led to the formation of a larger number of intermolecular heterotypic junction zones. Such shear-induced intermolecular association has previously been postulated to explain shear-induced viscosity increases (Kjøniksen et al., 2005; Matia-Merino, Goh, & Singh, 2012). Since similar shear thickening also occurs in the presence of hydrophilic CNCs, it appears that these CNCs do not interfere with the interaction between the gums. However, hydrophobized CNCs compete for binding sites and prevent the formation of additional junction zones under the shear. As a result, hybrid hydrogels with hydrophobized CNCs did not exhibit a shear-thickening region (Figure 2c,d). Some insights into structuring of complex fluids can be obtained from applying the Cox-Merz rule (Equation 1), which states that the apparent viscosity (η) and the magnitude of the complex viscosity (η^*) generated from steady shear and oscillatory shear experiments superimpose at a given shear rate ($\dot{\gamma}$) and frequency (ω):

$$|\eta^*(\omega)| \approx \eta(\dot{\gamma}) \quad (1)$$

where $\omega = \dot{\gamma}$. As can be seen from Figure 2c and d, LBG/XG gels followed the Cox-Merz rule. However, both types of hybrid gels deviated from Cox-Merz rule with complex viscosities being notably higher than the viscosities obtained in steady shear flow. This is characteristic of structured fluids. Oscillatory deformations performed at small amplitude do not affect the transient hydrogel structure, whilst larger deformations in steady flow cause structural distortions. Therefore, viscosities obtained from steady flow are lower than the complex viscosity. However, these structured fluids responded differently to shearing. Hybrid hydrogels with hydrophobized CNCs had higher viscosities in the region of Newtonian flow but dropped below the viscosities of hybrid hydrogels with non-modified CNCs in the shear-thinning region. Thus, rheological experiments imply that the network structures are different for hybrid hydrogels in the presence of nonmodified and hydrophobized CNCs.

3.3. *Effect of CNCs on emulsion gels based on synergistic LBG/XG gel.*

Emulsion gels are gelled systems containing dispersed droplets of an immiscible fluid (Dickinson, 2012; Lu, Mao, Hou, Miao, & Gao, 2019). Polysaccharides are used in emulsion

gels mainly as gelation agents. However, due to their low interfacial activity, additives of emulsifying agents are usually required for polysaccharide-based emulsion gels. Stable, self-supporting emulsion gels were obtained using CNC-free LBG/XG gels with total gum concentrations as low as 0.4 wt.% as well as with hybrid gels containing either hydrophilic, or hydrophobic, CNCs (Figure S9). The microstructure of these gels was probed using MCLSS and the distribution of the oil droplet volumes determined (Figure 4 and Table S1). It is apparent from the box plots (Figure 4a) that increasing the concentration of hydrophobic domains, either by increasing the LBG/XG concentration or via the introduction of hydrophobized CNCs, results in a decrease in the absolute droplet volumes. For example, the median droplet volume value decreases from 1405 to 904 μm^3 as the LBG/XG concentration increases from 0.4 to 0.6 wt.% and decreases to 241 μm^3 upon introduction of hydrophobic CNCs. By fixing the population category ranges, in this case the values determined for the 0.6X20 sample, it is possible to compare the population distribution between individual samples (Figure 4b) and sample categories (Figure 4c and 4d). Whilst an increase in the gum concentration decreases the absolute droplet volume, no significant change is observed in the population distribution (Figure 4c). In contrast, the addition of hydrophobized CNCs both decreases the absolute droplet volume and significantly increases the number of droplets that fall into the small population category (Figure 4d). The addition of hydrophilic CNCs, with minimal hydrophobic domains, makes no significant difference.

Figure 4. (a) Box plots comparing distribution of oil droplet volume between emulsion gel samples. Droplet volumes are divided into four categories: small (blue boxes), medium (red boxes), large (green boxes), and outliers (black diamonds). The 0.4X20 sample has a second medium category (orange box). Aggregates $< 3 \mu\text{m}^3$ are ignored for analysis. $n = 300$. (b) Oil droplet volume population distributions for each sample. Volume limits for each category defined by 0.6X20 sample. For all categories $N = 3$. Average oil droplet volume population distribution for (c) gum concentration ($N = 3$) and (d) gel modification ($N = 2$). Aggregate categories: small, $3 \leq X < 1443 \mu\text{m}^3$ (blue bars); medium, $1443 \leq X < 6840 \mu\text{m}^3$ (red bars with light spot scattering), large $6840 \leq X < 156623 \mu\text{m}^3$ (green bars with medium spot scattering), and outliers $X \geq 156623 \mu\text{m}^3$ (yellow bars with heavy spot scattering). † $p < 0.05$ compared with unmodified samples in the respective size category. ‡ $p < 0.05$ compared with CNC samples in the respective size category. Error: $\pm$ SE.

From the MCLSS data, it is apparent that corn oil autofluoresces under excitation at 405 nm (Figure 5, Figure S6 and Figure S10). This is most likely due to the ester groups present in the glycerol backbone, the carboxyl form of which have been previously associated with fluorescent n, π^* transitions involving free electron pairs in oxygen groups (Johns, Lewandowska, Green, & Eichhorn, 2020; Li, Li, An, Chen, & Xiao, 2019; Xu et al., 2019). Spectral maps of the samples (Figure 5 and S8) reveal that both hydrophilic and hydrophobized CNCs concentrate at the oil droplets as evidenced by an increase in the CNC map intensity in the same locations as an

increase in the corn oil map intensity. Due to the technique used to generate the 2D map from the 3D data it is not apparent from the frontal projection images (Figure 5a) as to whether the CNCs are present in the aqueous, or oil, phases. However, the transverse maps (Figure 5b) suggest that the hydrophilic CNCs are in the aqueous phase, given their presence throughout the image, whilst the hydrophobized CNCs are confined to the oil phase. It is also worth noting that the gums appear to interact with the oil phase, as evidenced by the corresponding increase in intensity, in both the CNC-free and hydrophilic CNC samples (Figure 5ai,ii and Figure S10a-d), which is expected due to the presence of hydrophobic domains within their structure. However, no clear increase in the gum intensity is observed in the hydrophobized CNC samples (Figure 5aiii and Figure S10e-g). This suggests that either no preferential interaction occurs between the oil and gums, which is unlikely given that the hydrophobized CNCs associated with the gums in the gel state (Figure 3c,d), or the majority of the oil droplets are at a scale below that detectable at the magnification used resulting in an apparent homogenous distribution. This second theory is supported by the increased oil intensity across the map in the hydrophobized CNC samples (Figure 5aiii and Figure S10e-g) and by the apparent decrease in the total detected volume of oil in the droplet volume analysis (Figure S11).

To further understand the effect of the presence of the different CNCs on the properties of the emulsion gels, the thermal response of the viscoelastic properties was investigated. In line with rheology of LBG/XG base and hybrid gels, the elastic modulus dominated over loss modulus for the emulsion gels across the temperature range (Figure 6a). The viscoelastic moduli of the CNC-free emulsion gels decreased at ~50 °C, as previously observed for the gel systems (Figure 2a,b), reflecting the aqueous continuous phase transition from a gel to a viscous solution. However, in the presence of both types of CNC, minimal variation was observed between 25-70 °C, despite changes being observed in the gel samples. This suggests that the interaction between the CNCs and the oil phase results in thermal stabilisation of the system, which could be due to localised increases in the CNC concentration around the oil phase resulting in stable gel phases. Similar behaviour was observed for CNC-free and hydrophobized CNC emulsion gels containing 40 wt.% of oil (Figure S12). However, phase separation was observed for the hybrid gel with hydrophilic CNCs, suggesting that these CNCs have a detrimental effect on the formation of emulsion gels. Further evidence for this is observed in the increase in the maximum oil volume in the large droplet category compared to the unmodified system (Figure 4a) and the slight, although not significant, decrease in the droplet distribution small population category (Figure 4c).

Figure 5. (a) Representative MCLSS frontal projection images of (i) 0.4X20, (ii) 0.4CNC20 and (iii) 0.4C₈CNC20 samples. All scale bars represent 200 μm . (b) Representative sections of MCLSS transverse projection spectrum maps of 0.4CNC20 and 0.4C₈CNC20 samples in (a), maps from top to bottom: LBG/XG, CNC, corn oil, combined. Sample maps are intensity maps of the corresponding spectrum. Spectra and larger images available in SI (Figure S6, Figure S10). Scale bar represents 100 μm .

Figure 6. (a) Temperature dependence of viscoelastic moduli for emulsion gels. Angular frequency 6.28 rad s^{-1} , strain amplitude 1.5 %. (b) Frequency sweeps for emulsion gels. Temperature 25 $^{\circ}\text{C}$, strain amplitude 1.5 %. Samples: 0.4X20 (black circles), 0.4CNC20 (blue hexagons), 0.4C₈CNC20 (red pentagons). G' (solid symbols), G'' (open symbols). Curves in (b) are the average of three measurements (Note: the error bars are smaller than the symbols).

Frequency sweeps confirmed the solid-like behaviour of the emulsion gels with G' higher than G'' over the used frequency range (Figure 6b). The introduction of CNCs increased the storage moduli, with hydrophobized CNCs having a greater effect. This is typical for emulsion gels; stronger gelled continuous phases generally yield stronger emulsion gels (Dickinson, 2012; Geremias-Andrade, Souki, Moraes, & Pinho, 2016). However, whilst all the gels observed a greater increase in G'' compared to G' with increasing angular frequency, the addition of hydrophilic CNCs made the emulsion gel less resilient, as evidenced by a higher initial $\tan(\delta)$ and a greater increase in its value over the angular frequency range (Figure S13), whilst the addition of hydrophobized CNCs increased the resilience compared to the CNC-free system.

It is important to consider that the rheology of a continuous phase is not the only factor that defines properties of emulsion gels. Emulsion gels can essentially be considered as composite materials. Therefore, the interaction of the dispersed phase with the continuous phase, the dispersed phase droplet size, and their spatial distribution will also influence the mechanical properties of the emulsion gels. The mechanical properties of the droplets depend on their size due to the Laplace pressure arising from surface tension (Dickinson, 2012). The storage modulus of liquid droplet is defined using the equation

$$G' = 2\gamma/r \quad (2)$$

where γ is the interfacial tension and r is the radius of a droplet. The median droplet radius across all samples investigated is 5.5 μm . Then, using the value of interfacial tension of corn oil as 25 mN m^{-1} (Ogino & Onishi, 1981) with the assumption that the CNCs and gums do not impact the oil-water interfacial tension, the average corn oil droplet storage modulus is expected to be ~10 kPa . This will increase as the droplet size decreases – the median observed droplet for the 0.4C₈CNC20 sample is 3.9 μm . For comparison, the equivalent G' of the LBG/XG gel samples are between 10-200 Pa (Figure 2). Thus, the oil phase is around an order of magnitude stiffer than the gelled aqueous matrices used in this study and may contribute to the stiffness of the emulsion gels. Comparison of the storage modulus of the emulsion gels (G'_E) to the storage modulus of the corresponding gel matrix (G'_M) (Figure 7) reveals significant differences dependent on the choice of CNC addition. For the pure LBG/XG polymer network, addition of the oil phase increases G' approx. 1.7 times for both 20 and 40 wt.% oil. This suggests that, whilst there is some interaction between the two phases, the oil droplets are effectively an inactive filler in terms of rheological properties with minimal reinforcing effect. However, the addition of hydrophilic CNCs completely removes any interactive effect between the two phases

with no observable difference between G'_E and G'_M . Given that the oil droplet distribution is not significantly different from the system with no CNCs present (Figure 4, Figure 5, Table S1, Figure S10, Figure S11), we theorise that the presence of CNCs at the interface between the two phases inhibits any interactive effect between the oil phase and the hydrophobic junctions in the LBG/XG polymer network, resulting in poor stabilisation of the oil droplets. This confirms that hydrophilic CNCs are not suitable additives for emulsion gel systems without the addition of surfactants. In contrast, the addition of hydrophobic CNCs significantly increases the storage modulus ratio compared to the other two samples, with further significant increases as the oil content is increased from 20 to 40 wt.%. We theorise that there is a two-fold effect here. Firstly, the presence of hydrophobic CNCs results in a significant decrease in the oil droplet size (Figure 4, Table S1), which will increase the oil phase storage modulus (Equation 2). Secondly, the ability of the hydrophobic CNCs to interact with both the LBG/XG polymer network junction zones (Figure 3) and the oil phase (Figure 4) enables them to improve the interfacial interaction between the two phases. As the oil content is increased the total interface area also increases, enhancing the effect.

Figure 7. Influence of oil content on the ratio between the storage modulus of the emulsion gel (G'_E) with respect to storage modulus of the corresponding gel matrix (G'_M). Oil content: 20 wt.% (no pattern), 40 wt.% (diagonal pattern). Total gum concentration: 0.4 wt.%, CNC concentration: 1.0 wt.%. Oscillation conditions: angular frequency 6.28 rad s⁻¹, strain amplitude 1.5 %, temperature 25 °C. Error: $\pm$ SE. * $p < 0.05$ between oil contents of 20 and 40 wt.%, † $p < 0.05$ compared to unmodified system ‡ $p < 0.05$ compared to CNC modified system.

4. Conclusions

The synergistic effect between LBG and XG is well known in the formation of gels. Here, we demonstrated that the addition of hydrophobized CNCs induced further enhancement in synergistic gel rheological properties. This effect of hydrophobized CNCs is superior compared with additives of hydrophilic CNCs despite the fact that rheological properties of individual gum solutions were not much different in the presence of both CNC types. Hydrophobized CNCs preferentially interacted with the hydrophobic junctions present in the LBG/XG network, resulting in a heterogeneous distribution, whilst the unmodified, hydrophilic CNCs were homogeneously distributed throughout the gel. As such, the hydrophobized CNCs elicited a greater change in the rheological response. Furthermore, hydrophobized CNCs had a positive impact on the structural and rheological properties of emulsions formed with corn oil. Hydrophilic CNCs appeared to be detrimental to the stability of the emulsion gels. It is theorised that their presence at the oil/water interface disrupted the interaction between the LBG/XG network and the oil, which resulted in poor performance. In contrast, the addition of hydrophobized CNCs resulted in significantly smaller oil droplets that were more homogeneously distributed compared to emulsion gels without CNCs or with hydrophilic CNCs. This suggested that hydrophobized CNCs were able to mediate interactions between the two phases. This work demonstrates the potential of amphiphilic nanocellulose as structuring and reinforcing additives in complex polysaccharide systems. Such nanoparticles can be exploited in combination with various hydrocolloids for the designing water-based soft materials.

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