Synergistic Stabilization of Emulsions and Emulsion Gels with Water-Soluble Polymers and Cellulose Nanocrystals

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ⓢ Supporting Information

ABSTRACT: The effect of water-soluble polymers on the properties of Pickering emulsions stabilized by cellulose nanocrystals (CNCs) was investigated. Pretreatment of CNCs with excess adsorbing polymer, hydroxyethyl cellulose (HEC) or methyl cellulose (MC), gave smaller and more stable dodecane-in-water emulsion droplets compared to either polymer or CNCs alone, i.e., synergistic stabilization. By contrast, dextran, which does not adsorb on CNCs, gave unstable emulsions, with or without CNCs. CNCs with HEC or MC produced emulsions that showed no significant creaming or phase separation over several months. Interfacial tension, quartz crystal microbalance and confocal laser scanning microscopy measurements indicate that both HEC and MC are surface active and adsorb onto CNCs. 75% of the oil–water interface is covered by CNC particles coated with HEC or MC and the remaining interface is stabilized by HEC or MC chains not bound to cellulose. Viscoelastic emulsion gels were also produced by adding excess MC to the CNC-HEC emulsions and heating above 70 °C. The thermogelation was reversible, and multiple cycles of heating/cooling did not lead to coalescence of the emulsion. This work points to broad application of CNCs with water-soluble polymers as promising green emulsion stabilizers for food, pharmaceutical, and cosmetic products.

KEYWORDS: Cellulose nanocrystals, Pickering emulsions, Confocal microscopy, Interfacial tension, Emulsion gels, Hydroxyethyl cellulose, Methyl cellulose
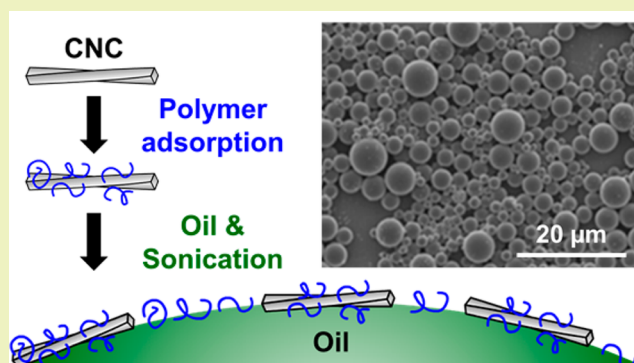
INTRODUCTION

Emulsions are of great practical interest due to their widespread use in the formulation of food, pharmaceutical, cosmetic, paint, and ink products. In many of these applications, the stability of emulsions is of utmost importance to retain their desired properties over long time periods. Addition of surface active molecules like surfactants, amphiphilic polymers, lipids, and proteins allows the formulation of stable emulsions due to the formation of structured interfacial films. Alternatively, Ramsden[1] and Pickering[2] demonstrated that solid colloidal particles may adsorb at interfaces to form "Pickering emulsions". It is postulated that the partitioning of colloidal particles at the oil–water interface produces a densely packed particle layer and thus prevents both emulsion flocculation and coalescence by inducing a steric barrier. Various inorganic and organic colloidal particles with different sizes, shapes and surface chemistries have been employed to prepare Pickering emulsions, including silica,[3] clay,[4] carbon nanotubes,[5] magnetic particles,[6] gold nanoparticles,[7] polymeric microgel particles,[8] and cellulose nanocrystals.[9]

Cellulose nanocrystals (CNCs) from cotton and wood are highly crystalline rigid rod-shaped nanoparticles that are 5–10 nm in cross section and 100–200 nm long.[10] CNCs are entering the marketplace in commercial scale quantities in Canada, the United States, and Europe.[11] They are most commonly prepared by sulfuric acid hydrolysis, which gives nanocrystals with anionic sulfate half-ester groups on their surface.[10] Capron and co-workers have established the groundwork for using CNCs as emulsion stabilizers.[9,12−14] They found that unmodified CNCs are effective at stabilizing emulsions when they have a low surface charge density, or when the surface charge is screened in the presence of salt. In their work, 84% surface coverage of the emulsion droplets was required to impede droplet coalescence with cotton-derived CNCs.[13] However, a surface coverage smaller than 40% was sufficient to produce stable emulsions when CNCs with higher aspect ratios (from bacterial cellulose) were used. Capron et al. also prepared high-internal-phase emulsion "gels" by making CNC-stabilized emulsions with equal parts oil and water and then slowly increasing the oil content.[14]

Because many commercial emulsion-based products contain polymers, surfactants, and particles, the characterization of emulsions prepared with all of these components, and a better understanding of emulsion stabilization mechanisms, are of great importance. Studying the interfacial properties of cellulose nanocrystals in the presence of interacting surfactants and

polymers can provide useful insight into the mechanistic origin of some emulsion properties. In our previous work, we investigated in situ surface modification of CNCs with cationic surfactants and demonstrated that surfactant adsorption changed the hydrophobicity of CNCs and could be used to tailor CNC Pickering emulsions from oil-in-water (o/w) to water-in-oil (w/o).[15] In another literature example, thermoresponsive poly(N-isopropylacrylamide) (PNIPAM) brushes were grafted from CNCs to control the hydrophobicity with temperature to tune the emulsion stability.[16] The emulsions stabilized by PNIPAM-g-CNCs broke after heating above the lower critical solution temperature (LCST) of PNIPAM, where the temperature responsiveness of the brushes imparted temperature responsiveness to the CNC Pickering emulsifier and thus the emulsion overall. Similarly, a weak polyelectrolyte poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) was grafted from the surface of CNCs providing both pH and temperature sensitivity to the emulsions.[17] Unlike unmodified cellulose nanocrystals, grafted CNCs significantly reduced surface and interfacial tension leading to enhanced emulsion stability and the ability to trigger emulsification and demulsification of oil droplets.[16,17]

Chemical modification of nanoparticles through covalent chemistry and polymer grafting are generally lengthy, "ungreen", and costly methods to modify surface properties, particularly for CNCs, which are only stable in aqueous solutions with low ionic strength. Physical adsorption of polymers onto particles to tune hydrophobicity and interfacial tension offers an easier route. Saleh et al.[18] demonstrated that physisorbed layers of amphiphilic triblock copolymers not only improved the colloidal stability of iron nanoparticles in water but also drove them to adsorb at the oil–water interface, forming highly stable emulsions. Similarly, Feng et al.[19] used ionic interactions between carboxylic acid groups on single-walled carbon nanotubes (SWCNTs) and amine groups on amine-terminated polystyrene to facilitate the assembly of SWCNTs at the oil–water interface and produce stable emulsions. By using the interactions between calcium ions on hydroxyapatite nanoparticles and carboxyl end groups on polystyrene chains, Okada et al.[20] found that the presence of polymer facilitated the adsorption of hydroxyapatite at the oil–water interface and contributed to the stabilization of hydroxyapatite-based Pickering emulsions. However, to the best of our knowledge, no CNC Pickering emulsion work has employed the physical adsorption of water-soluble polymers to control the emulsifying properties of CNCs.

We use hydroxyethyl cellulose (HEC) and methyl cellulose (MC), which are commercial nonionic water-soluble cellulose derivatives that are ubiquitous as gelation agents, stabilizers, and binders in cosmetics, pharmaceuticals, polymerization processes, oil exploitation, food, textiles, papermaking, paints, and many other industrial applications.[21] The adsorption of cellulose derivatives to cellulose in the form of pulp fibers and nanocellulose has been well-documented,[22–24] and recently, we reported on the strong adsorption of HEC, hydroxypropyl guar, and locust bean gum to CNCs.[25] This adsorption is a prerequisite for the extremely stable emulsions produced in this work.

Besides liquid emulsions, it is also common to find formulated products that are structured o/w emulsions that are more gel-like soft solids. These viscoelastic emulsions can be of two types: (1) where water is the continuous phase, with emulsifiers and added water-soluble gelation agents; or (2)

where viscous concentrated emulsions are formed from the close crowding of dispersed oil droplets, i.e., where the internal phase volume fraction is 0.74 or greater, high internal phase emulsions.[26] However, high internal phase emulsions using solid particles as stabilizers require many steps to prepare, or involve particulate emulsifiers with specific hydrophobicity, which makes them nonideal in commercial productions.[14,27] Centrifugation is sometimes used to force oil droplets together and generate a concentrated emulsion phase with gel-like behavior,[28] however, centrifugation can be detrimental to the stability of emulsions and is a nontrivial process at the industrial scale.[9] Therefore, we present an industrially relevant process to obtain emulsion gels using CNCs with polymers which act as both emulsion stabilizers and thickening agents for the aqueous phase. This work is anticipated to lead to new and interesting applications of emulsions and emulsion gels, where using biobased materials (and moving away from synthetic surfactants) is advantageous.

■ EXPERIMENTAL SECTION

Materials. Methyl cellulose (MC, 40 kDa, degree of substitution of 1.6–1.9), 2-hydroxyethyl cellulose (HEC, 90 kDa, molar substitution of 2.5), dextran (DEX, 70 kDa), dodecane, corn oil, Nile red, Nile blue, 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF), azobis-(isobutyronitrile) (AIBN), and sodium chloride were all purchased from Sigma-Aldrich. All water used was deionized and further purified with a Barnstead Nanopure Diamond system (Thermo Scientific).

Preparation of Cellulose Nanocrystals. CNCs were prepared by sulfuric acid hydrolysis of cotton from cotton filter aid (Whatman ashless filter aid, GE Healthcare Canada), as described previously.[29] Briefly, 40 g of the filter aid was treated with 700 mL of 64 wt % sulfuric acid (Fischer Scientific) for 45 min at 45 °C. The hydrolysis reaction was quenched with cold water and acid/degraded sugars were removed by centrifugation and extensive dialysis against purified water. The surface charge density of sulfate half-ester groups on CNCs was determined by conductometric titration to be 0.33 ± 0.02 e/nm^2, with average crystal dimensions of 128×7 nm from transmission electron microscopy (TEM) image analysis (see the Supporting Information, Figure S1).

Preparation of Emulsions. Emulsions of 6 mL containing equal volumes of dodecane and aqueous dispersions (1:1 oil to water) were obtained by mixing 3 mL of dodecane with 1.5 mL of 0.6 wt % CNCs and 1.5 mL of 0.4 wt % polymer solution, making the final concentration of CNCs and polymer in the emulsions 0.3 and 0.2 wt %, respectively. The mixture was immediately emulsified using a probe sonicator (Sonifier 450, Branson Ultrasonics), in an ice bath for 1 min at an intensity level 6 and 50% pulses for every emulsion, which corresponds to an energy input of approximately 1371 J/g of emulsion. The polymer solutions tested were MC, HEC, and DEX. The emulsions using both CNCs and polymers were denoted as CNC-MC, CNC-HEC, and CNC-DEX emulsion, respectively. For comparison, emulsions using either CNCs or polymers as the only emulsifier were prepared. In all samples, 50 mM NaCl was maintained in the water phase.

Visualization of Emulsion Droplets by SEM. Following the work of Kalashnikova et al.,[9] we polymerized the internal emulsion oil phase as a way to visualize the emulsion droplets under scanning electron microscopy (SEM). Solid polystyrene particles were prepared through polymerization of styrene emulsion droplets stabilized by CNCs alone or in the presence of polymers. Aqueous suspensions containing 0.3 wt % CNC were sonicated for 1 min and degassed with nitrogen for 3 min. Styrene was mixed with AIBN, an oil-soluble initiator, to obtain a styrene/AIBN ratio of 100/1 (w/w). A total volume of 3 mL of this mixture was added to equal volume of the CNC suspension, and an emulsion was produced by sonication for 1 min. In some samples, 0.2 wt % HEC or MC was used together with 0.3 wt % CNC in stabilizing the emulsions. The emulsions were placed

in oven at 65 °C, without stirring, for 8 h. The dried polystyrene particles were metallized with 5 nm Pt and visualized with a JEOL 7000F SEM (JEOL Ltd., Japan). Micrographs were taken at 6 mm working distance with 20 kV acceleration voltage.

Fluorescent Labeling. The CNCs were labeled with Nile blue. Nile blue (10 mg) was added to 50 mL of 1 wt % CNC suspension (pH 3) and mixed for 24 h in the dark. Unbound Nile blue was removed by extensive dialysis against purified water. The polymers were labeled with DTAF as previously described for CNCs.[30] HEC or MC (500 mg) was reacted with DTAF (7.5 mg) under alkaline conditions (0.2 M NaOH) for 24 h, in the dark, with mechanical stirring. To remove unreacted DTAF and the bulk of the NaOH, extensive dialysis against purified water was employed. Purified reaction products were freeze-dried and stored in the dark. The labeled CNCs and polymers are denoted as CNC-Nile blue, HEC-DTAF, and MC-DTAF, respectively.

Confocal Laser Scanning Microscopy (CLSM). CLSM was use to investigate the microstructure of the emulsions; the oil phase was stained with Nile red (492/520 nm). For some samples, the CNCs and polymers were labeled with Nile blue (630/660 nm) and DTAF (495/517 nm), respectively. Images were obtained with a Zeiss LSM 510 Meta on an Axiovert 200 M microscope (Zeiss, Gottingen, Germany). For high resolution images, a 63× water-immersion laser technique was used.

Malvern Mastersizer. A Malvern Mastersizer 2000G instrument with a HeNe laser operating at 633 nm was used to measure the emulsion droplet size. The mean droplet diameter was taken to be the volume mean diameter ($D_{4/3}$) from 3 replicate measurements. It is mathematically expressed as $D_{4/3} = \sum D_i^4 N_i / \sum D_i^3 N_i$, where D_i is the droplet diameter for droplet fraction i, and N_i is the number of droplets with size D_i. The "span" was reported as a measurement of the emulsion droplet diameter size distribution and calculated using span $= (D(0.9)-D(0.1)/D(0.5)$, where $D(0.9)$ is the droplet diameter for which 90% of the droplets are below this size, $D(0.5)$ is the droplet diameter for which 50% of the droplets are smaller than this size, and $D(0.1)$ is the diameter for which only 10% of the droplets lie below this size. The stability of emulsions was determined by following the emulsion droplet diameter over 150 h.

Surface Coverage Calculation. The percentage of emulsion droplet surface area covered by CNCs was calculated, as derived previously.[9] The surface coverage C is given by the ratio of the theoretical maximum projected surface area of the emulsifying particles to the total surface area of the emulsion droplets:

$$C = \frac{mD}{6h\rho V} \tag{1}$$

where m is the mass of CNCs, D is the $D_{4/3}$ mean diameter of the emulsion droplets, h is the CNC thickness (7 nm as determined by TEM), ρ is the CNC density (1.59 g/cm³),[31] and V is the volume of oil used in the emulsion.

Interfacial Tension. Interfacial tension as a function of time was measured by the pendant drop method with a Krüss Drop Shape Analysis System DSA10. Prior to the measurement, a sample (ca. 50 mL) of dodecane was added to a glass box of 5.0 (L) × 5.0 (W) × 5.0 (H) cm, and a pendant drop of CNC suspension and/or polymer aqueous solution of about 20 μL, was formed on the end of a stainless steel needle immersed in the dodecane oil phase. The aging of the interface can be accurately monitored by measuring the interfacial tension of a drop having a constant interfacial area over 20 min. Typically, the data collected after 20 min aging were used as the equilibrium interfacial tension values. Three replicate measurements were done for each sample and the data were averaged. Error bars represent the standard deviation of the mean.

Rheology. Oscillatory rheological measurements of emulsions were performed using an ARES rheometer (TA Instruments) with parallel-plate geometry. A 7 mm diameter parallel plate with a 1.5 mm gap distance was used. Dynamic frequency sweeps were performed at 1% strains, which were confirmed to be in the linear viscoelastic range for each sample, from 0.1 to 100 rad/s to determine the storage modulus (G') and loss modulus (G'') of the emulsions. Measurements

were done at temperatures of 25 and 70 °C. The evaporation of water from the samples was avoided by covering the sides of the plate with low viscosity mineral oil. After heating samples to 70 °C, they were allowed to thermally equilibrate for 3 min before the measurements.

■ RESULTS

Emulsion Droplet Size. As most formulated chemical products are complex mixtures of surfactants, polymers, and particles in relatively high ionic strength solutions, we herein report the production of CNC-stabilized Pickering emulsion in the presence of water-soluble polysaccharides with a background salt concentration of 50 mM. Experimentally, HEC, MC, and dextran polymers were first mixed with CNCs, an equal volume of oil was added, and the mixture was emulsified through ultrasonication. For the CNC and polymer concentrations used here, we note that no gelation occurred prior to emulsification, with or without 50 mM NaCl, as we are substantially below the polymer overlap concentrations and CNC with adsorbing polymer gelation has not been observed at such low CNC concentrations.[25] To investigate emulsion droplet size, the oil phase (dodecane) was stained with an oil-soluble fluorophore, Nile red, and was visualized by confocal laser scanning microscopy (CLSM, Figure 1). The continuous phase was not stained and appears black.
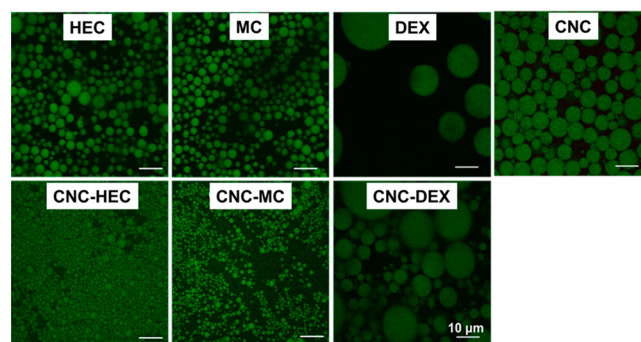

Figure 1. Confocal laser scanning microscopy images of 1:1 dodecane–water emulsions stabilized by 0.3 wt % CNCs and 0.2 wt % polymers, either individually (top) or together (bottom). The combination of hydroxyethyl cellulose or methylcellulose with CNCs synergistically stabilizes smaller emulsion droplets. The oil phase is dyed with Nile red and appears green in the images. All scale bars are 10 μm.

CNCs partitioned at the oil–water interface and led to stable o/w emulsions, as shown previously.[12,15] The mean diameter of oil droplets stabilized by CNCs alone is 12 μm, whereas the samples prepared with 0.2 wt % HEC or MC as the only emulsifier have droplets with mean diameters of 6 μm (Figure 1, top row). Dextran, on the other hand, displayed poor emulsifying capability, giving emulsion droplets of 28 μm. In all cases, the combination of CNCs and polymers gave emulsions with smaller oil droplets than the polymers alone (Figure 1, bottom row), which is important, as it relates to enhanced emulsion stability overall.

Our previous work demonstrated that HEC adsorbs on CNC surfaces and increases the viscosity of CNC-HEC mixtures due to an increase in the overall particle volume fraction.[25] Adsorption of MC on CNC surfaces was also studied by quartz crystal microbalance gravimetry and similar adsorption behavior was observed (see the Supporting Information, Figure S2). The CNC particle size increases due to polymer

adsorption, and is estimated as being a 10 nm thick coating as determined previously by surface plasmon resonance spectroscopy and dynamic light scattering.[25] As shown in Figure 1, by adding HEC or MC to CNC suspensions before emulsification, smaller emulsion droplets with mean diameters of about 3 μm were obtained, demonstrating the synergistic effect of adding adsorbing polymers to CNCs. The term "synergy" is used in recognition of the fact that the overall effect of premixing HEC or MC and CNCs, on emulsion droplet size, is more than would be anticipated from simply combining two emulsifiers. On the other hand, dextran does not adsorb to CNCs[25,32] and the combination of dextran and CNCs produced emulsions with relatively larger droplets (21 μm).

Droplet size was corroborated by SEM imaging; liquid emulsions cannot be imaged directly by SEM so a method where dodecane is replaced by styrene and polymerized,[9] was employed (Figure 2). CNCs (0.3 wt %) alone led to polystyrene particles with average diameters of 20 μm. The addition of HEC or MC greatly reduced the polystyrene particle diameters to 2−3 μm.
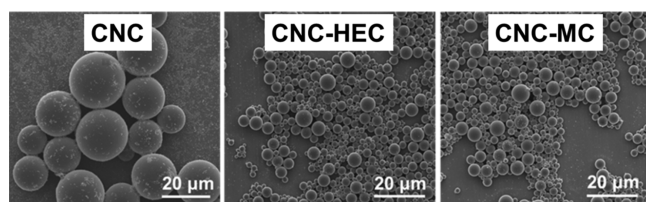

Figure 2. Scanning electron microscopy images of polymerized styrene emulsion droplets stabilized by CNCs alone (CNCs: 0.3 wt %) or in the presence of HEC (CNCs, 0.3 wt %; HEC, 0.2 wt %) or MC (CNCs, 0.3 wt %; MC, 0.2 wt %). All scale bars are 20 μm.

In addition to the microscopy images, emulsion droplet size and size polydispersity were quantified by Malvern Mastersizer measurements. The concentration of CNCs in the water phase was varied for a fixed oil−water volume ratio of 1:1. For the emulsions containing both CNCs and polymer as emulsifiers, the mass ratio of CNCs:polymer was fixed at 3:2. Mean droplet diameter and span results for emulsions stabilized by CNCs alone and together with each polymer are given in Table 1. For CNC-only emulsions, increasing the CNC concentration resulted in decreasing the mean droplet diameter before a plateau value of about 11 μm was reached at 0.4 wt % CNCs. Higher concentrations of CNCs provide larger interfacial area which leads to the decreasing droplet size. A similar trend is

observed for the emulsions stabilized by both CNCs and HEC/MC. However, in the presence of dextran and CNCs, larger emulsion droplets with a broader size distribution are obtained. These droplet size results follow those observed by CLSM and SEM and furthermore show the degree of control possible by varying the amount of emulsifier, even within a small concentration range.

The "synergistic" effect of using both CNCs and polymers as emulsion stabilizers is exemplified by comparing the mean oil droplet diameter for emulsions stabilized by 0.5 wt % CNCs, 0.5 wt % polymers, or 0.3 wt % CNCs + 0.2 wt % polymers (data in Table 1 and footnote). Though the concentration of emulsifiers was the same for these three types of emulsions, the emulsions with both CNCs and adsorbing polymers produced smaller oil droplets. This suggests the occurrence of a synergistic stabilization mechanism for the emulsions with CNCs and adsorbing polymers like HEC and MC; these emulsions have smaller droplet sizes than would be expected from simply adding up the emulsifier concentrations (i.e., costabilization).

Emulsion Stability. The stability of dodecane−water emulsions stabilized by CNCs and polymers was assessed by monitoring the change in mean droplet diameter over time using a Malvern Mastersizer. CNC and polymer concentrations were fixed at 0.3 and 0.2 wt %, respectively. Figure 3 shows that
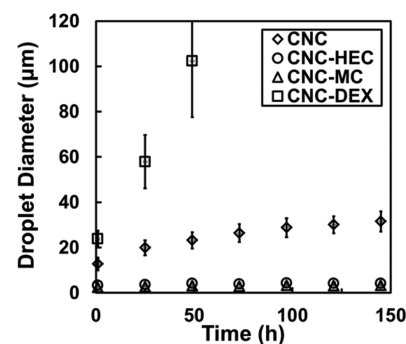

Figure 3. Mean droplet diameter of 1:1 dodecane−water emulsions stabilized by 0.3 wt % CNCs alone and in the presence of 0.2 wt % polymer over 150 h, as measured by a Malvern Mastersizer instrument. The droplet diameter was taken to be the volume mean diameter ($D_{4/3}$) from three replicate measurements and the data were averaged. Error bars represent one standard deviation of the mean.

Table 1. Summary of the Mean Emulsion Droplet Diameter and Span for 1:1 Dodecane−Water Emulsions Prepared with Varying CNC Concentration As Measured by Malvern Mastersizer Measurement[a]

CNC conc. (wt %)	CNC emulsion		CNC-HEC emulsion		CNC-MC emulsion		CNC-DEX emulsion	
	mean diameter (μm)	span[b]	mean diameter (μm)	span	mean diameter (μm)	span	mean diameter (μm)	span
0.05	58	2.6	15	2.2	12	2.3	102	3.7
0.1	27	2.3	7.2	2.0	6.2	1.9	52	3.5
0.2	18	2.1	4.5	1.9	3.3	1.7	29	3.3
0.3	13	1.8	3.3	1.5	2.4	1.6	18	3.4
0.4	11	1.9	3.1	1.7	2.2	1.6	15	3.5
0.5	10	1.7	2.8	1.5	1.9	1.5	13	3.3

[a]The mass ratio of CNC:polymer was fixed to 3:2. The polymers tested were MC, HEC, and DEX. The emulsions using both CNCs and polymers were denoted as CNC-MC, CNC-HEC, and CNC-DEX emulsion. Emulsions stabilized by 0.5 wt % HEC or MC alone were also tested and displayed mean diameters of 5.2 and 4.3 μm, respectively. A salt concentration of 50 mM was maintained by adding NaCl to the water phase. [b]The span is calculated as described in the Experimental Section.

the addition of HEC or MC to CNCs enables the production of emulsions with minimal droplet change over 6 days, suggesting the synergistic effects that HEC and MC have on stabilizing emulsions. Conversely, emulsion droplet sizes increased over the first day and then plateau for CNC-only emulsions but for CNC-DEX emulsions, droplet size increased considerably in the first 2 days (due to droplet coalescence) and then could not be measured because the droplets were beyond the instrument's detection limit and/or macroscopic phase separation occurred. Polymer-only stabilized emulsions also show significant coalescence and droplet sizes grow steadily over 6 days, surpassing 30 μm droplet sizes at the end of the testing period (see the Supporting Information, Figure S3).

The long-term stability of our emulsions was determined by visual inspection 30 days after preparation (see the Supporting Information, Figure S4). Emulsions stabilized by either CNCs or HEC show extensive creaming and color intensity decrease which imply the occurrence of coalescence. MC alone is a reasonable emulsifier exhibiting only mild creaming. Dextran alone, on the other hand, could not provide long-term stability to the emulsion and macroscopic phase separation was observed after 1 month of storage. Combining dextran and CNCs to prepare emulsions does not improve the emulsion stability either. However, both HEC and MC seem to greatly enhance the stability of CNC Pickering emulsions whereby only mild creaming was observed; in fact, one year later there is no obvious change in the appearance of these emulsions.

Location of CNCs and Polymers in the Emulsions. To determine the role of the various components in the emulsions, CNC particles and polymers were labeled with Nile blue and DTAF, respectively, and the emulsions were imaged by CLSM (Figure 4). Labeled CNCs and polymers are denoted as CNC-Nile blue, HEC-DTAF, and MC-DTAF and the labeling was confirmed with UV/Visible spectroscopy (see the Supporting Information, Figure S5). CNC-Nile Blue appears red in the original CLSM images (see the Supporting Information, Figure
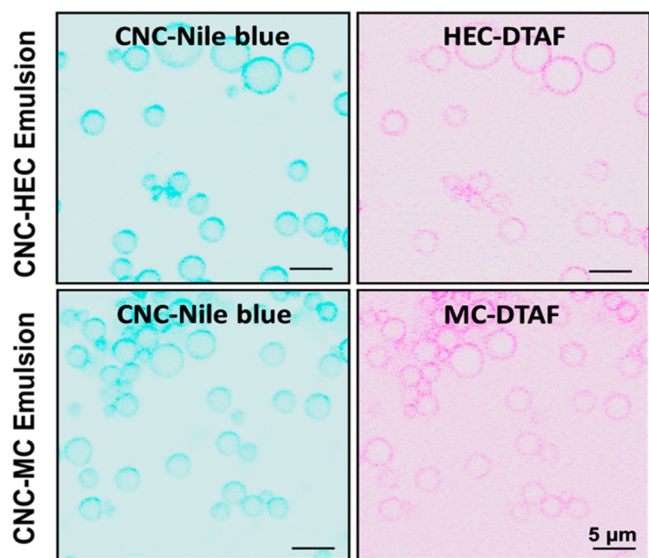
S6) and the ring structure suggests CNC particles are preferentially localized at the oil−water interface. On the other hand, HEC-DTAF and MC-DTAF appear green in the original CLSM images (see the Supporting Information, Figure S6) and display similar ring structures, which implies the partition of both HEC and MC at the interface. To obtain more visible distinction between areas, the original images have been color inverted using Adobe Photoshop and are shown in Figure 4. The presence of similar ring structures suggests the colocalization of both CNCs and polymers at the oil−water interface, implying that both emulsifiers are involved in stabilizing the oil droplets in water.

Interfacial Tension. Interfacial tension as a function of time was used to assess the adsorption of polymers and CNCs at the oil−water interface through pendant drop experiments. As shown in Figure 5a, dodecane−water interfacial tension was
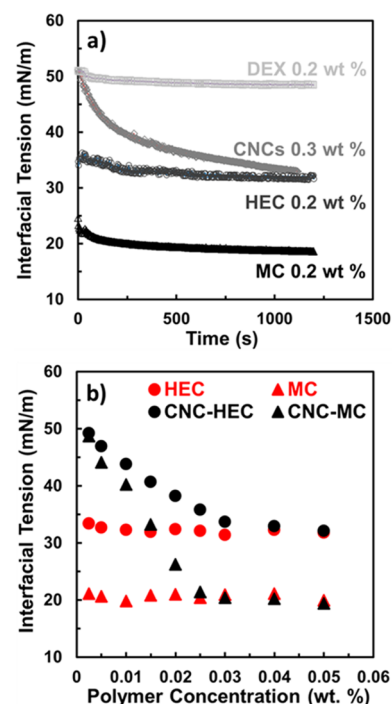

Figure 5. (a) Time dependence of dodecane−water interfacial tension for various polymers (0.2 wt % dextran, HEC, and MC) and CNCs (0.3 wt %); (b) interfacial tension (dodecane−water) with increasing HEC or MC concentration for either polymers alone (red symbols) or for the 0.5 wt % CNC-polymer systems (black symbols). Error bars are smaller than the filled symbols.

Figure 4. Confocal laser scanning micrographs of 1:1 dodecane−water emulsions stabilized by CNCs labeled with Nile blue and polymers labeled with DTAF (top, CNC-Nile blue and HEC-DTAF; bottom, CNC-Nile blue and MC-DTAF, the images have been color inverted using Adobe Photoshop). All scale bars are 5 μm.

measured over 20 min. CNCs lower the oil−water interfacial tension but even after 20 min has not reached equilibrium, implying the slow partitioning of CNC particles to the oil−water interface, likely due to their large hydrodynamic size.[33] HEC reduces the oil−water interfacial tension to an equilibrium value of 32 mN/m, in about 5 min, which indicates that HEC partitions at the interface and can form a polymer layer to stabilize emulsion droplets. MC is even more effective in reducing oil−water interfacial tension to an equilibrium value of 20 mN/m (in 5 min), meaning that MC is more surface active than HEC. However, the interfacial tension values of dextran display only a small reduction of about 1−2 mN/m from the starting value of 50 mN/m. Therefore, dextran is not surface active and not an effective emulsifier for stabilizing o/w emulsions.

Interfacial tension measurements for the combination systems, i.e., CNC-HEC and CNC-MC, with increasing polymer concentration are shown in Figure 5b. At low polymer concentrations, interfacial tension values of the samples with both CNCs and polymers are higher than for polymers alone, implying that polymers are preferentially adsorbed onto the CNC surface rather than the oil−water interface. The interfacial tension values decrease as polymer concentration is increased until a plateau value is reached. The minimum interfacial tension values are obtained when polymer concentrations are increased to values above 0.030 and 0.025 wt % for CNC-HEC and CNC-MC, respectively. These minimum interfacial tension values for CNC−polymer mixtures are close to the interfacial tension of polymers alone (Figure 5b).

Surface Coverage of Oil Droplets by CNCs. The maximum packing density of CNCs at the oil−water interface was calculated according to eq 1, for emulsions prepared with different CNC concentrations, using the mean droplet sizes reported in Table 1. These values are plotted against m, the mass of CNCs in the aqueous phase, in Figure 6. For emulsions
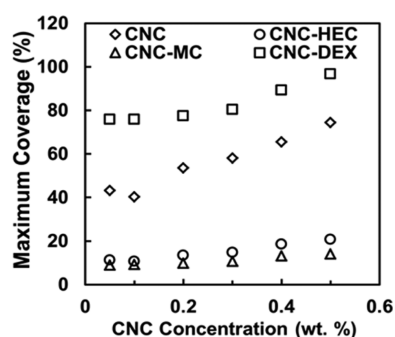

Figure 6. Maximum surface coverage vs CNC concentration (in the water phase) for 1:1 dodecane−water emulsions of CNC, CNC-HEC, CNC-MC, and CNC-DEX, calculated using eq 1.

stabilized by CNCs alone, surface coverage is increased at higher CNC concentrations, reaching a maximum coverage of 74% with 0.5 wt % CNCs. A similar trend of increasing surface coverage with CNC concentration is observed for emulsions stabilized by both CNCs and polymers, as well. However, the presence of HEC and MC seems to greatly reduce surface coverage to values below 20%. According to Capron et al.[13] 84% is the minimum CNC surface coverage needed for stable emulsions (with our current aspect ratio CNCs), which implies that HEC and MC are playing an important role to help stabilize the interface, thus requiring fewer CNCs.

Emulsion Gels. Many food, cosmetic, and pharmaceutical products are emulsion-based soft solids that display gel-like behavior.[26] The expression "emulsion gel" (or structured emulsions) is used to denote this class of material. Emulsion gels were prepared by adding excess MC to CNC-HEC emulsions. MC phase separates with heating; the cloud point temperature is in the range 50−60 °C,[34,35] depending upon the degree of substitution. At higher concentrations, phase separation results in fibril formation, giving gel structures.[36] In our work, 0.3 wt % CNCs, 0.2 wt % HEC with an oil volume fraction of 0.5 was used as the stock emulsion, and then equal volumes of 2 wt % HEC or MC were added for comparison purposes (giving a final oil fraction of 0.25).

CNC-HEC emulsions with added MC (denoted CNC-HEC +MC) show classic gel behavior where elastic modulus (G')

dominates over loss modulus (G'') (Figure 7a) and a flat frequency response at 70 °C, implying that gelation occurs. The
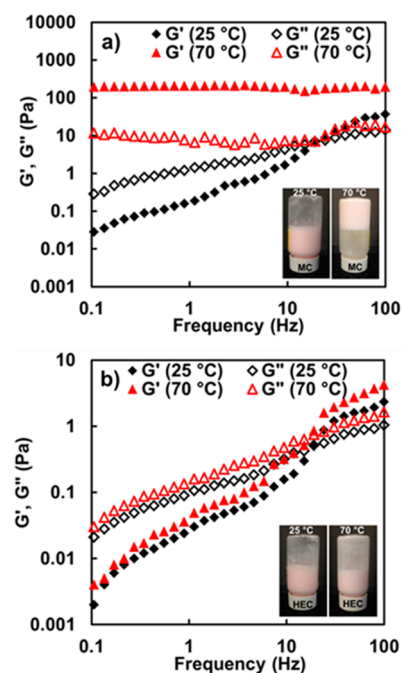

Figure 7. Storage modulus, G', and loss modulus, G'', of (a) CNC-HEC+MC emulsion (CNCs, 0.15 wt %; HEC, 0.1 wt %; MC, 1 wt %, 25 vol % o/w emulsion); (b) CNC-HEC+HEC emulsion, (CNCs, 0.15 wt %; HEC, 1.1 wt %; 25 vol % o/w emulsion) at 25 °C (black diamonds) and 70 °C (red triangles). Inset images show that gel formation was achieved only in the CNC-HEC+MC emulsion at 70 °C.

inset in Figure 7a demonstrates that a self-supporting gel is formed at high temperatures. Similarly, stock CNCs-HEC emulsions without additional polymers do not display viscoelastic behavior at 70 °C either. However, for CNC-HEC emulsions with added HEC (denoted CNC-HEC+HEC), the overall behavior remains reminiscent of viscous liquids at both 25 and 70 °C (Figure 7b) where G'' is larger than G'. There is no rheological difference between different temperatures. The inset in Figure 7b shows photographs of the emulsions at 25 and 70 °C confirming that emulsions flow similarly at both temperatures.

The thermoresponsive behavior of CNC-HEC+MC emulsions was fully reversible as tested over five cycles. No noticeable change of emulsion droplet morphology was observed (see the Supporting Information, Figure S7), which suggests that the emulsions are stable to coalescence in both heating and cooling processes. This is noteworthy given that temperature affects the physical properties of oil, water, and interfacial films, which control the emulsion stability.[37] Generally, temperature increases the frequency of droplet collisions, reduces the interfacial viscosity, and increases the film-drainage rate, which all result in faster droplet coalescence. Emulsions stabilized by MC alone also respond to temperature but the emulsions are inhomogeneous and exhibit fast creaming (data not shown) compared to the CNC-HEC+MC emulsion gels, which are uniform and stable.

DISCUSSION

HEC and MC adsorb to cellulose and are surface active (Figure 5a); this combination led to polymer-coated CNCs that provide enhanced stability to dodecane−water emulsions (Figure 3). Dextran does not adsorb to cellulose and is not significantly surface active, and thus CNC-DEX emulsions coalesced quickly. Importantly, the droplet sizes in the CNC-HEC and CNC-MC emulsions are small (Figures 1 and 2 and Table 1) and fluorescent labeling indicates that both CNCs and polymers are present at the oil−water interface (Figure 4), which leads to a synergistic stabilization mechanism.

This agrees with the literature that states that particulate emulsifiers alone normally do not lower interfacial tension significantly and, at best, their partitioning to the oil−water interface is diffusion limited.[38] HEC and MC, on the other hand, can reduce the oil−water interfacial tension to 32 and 20 mN/m, respectively, in under 5 min (Figure 5a) alone and when combined with CNCs. QCM-D adsorption measurements imply that HEC[25] and MC adsorption to cellulose is fast (see the Supporting Information, Figure S2) such that the vigorous mixing of CNCs and polymers before oil is added, and before ultrasonication treatment, is sufficient to have coated CNCs which act as improved emulsifiers.

HEC and MC preferentially adsorb to CNCs over the oil−water interface, thus the CNC:polymer ratio must be carefully chosen to optimize emulsion stability. The critical point where CNC particle surfaces are saturated with polymer and excess free polymer starts to partition to the oil−water interface was calculated based on the intersection point of the two linear regimes in the interfacial tension adsorption isotherms (Figure 5b). The maximum surface coverage of HEC and MC on CNC surfaces was found here to be 0.16 and 0.14 mg/m^2, respectively. It is interesting that these values are so similar and suggests that we can use interfacial tension isotherms to measure the adsorption capacity or apparent surface area of CNCs. Accordingly, CNC surfaces are saturated with polymers when the ratio of CNC:HEC and CNC:MC is smaller than about 20:1 (by mass). Because the mass ratio of CNC:polymer was fixed at 3:2 in this work, we postulate that all CNC particles have been coated with a polymer layer with about 90% of the added polymer present as free polymer.

The combination of polymer-coated CNCs and free polymer allows for small emulsion droplet sizes and the steric barrier from the Pickering emulsion properties appears to significantly limit droplet coalescence. Interestingly, however, the actual coverage of CNCs on the droplet surfaces is lower than previously reported.[9,12,13] Due to the small droplet sizes in CNC-HEC and CNC-MC systems (1.9−15 μm), the surface coverage by actual (uncoated) CNCs is less than 20% for a 1:1 dodecane−water emulsion with 0.5 wt % CNCs (Figure 6). Because MC is more surface active than HEC (Figure 5) and alone stabilizes smaller oil droplets than HEC (Figure 1, Table 1, and Figure S3 (Supporting Information)), the smallest CNC−polymer emulsion droplets were likewise obtained with MC.

The composition at the oil−water interface was estimated by assuming that all of the polymer-coated CNCs were adsorbed at the oil−water interface (confocal microscopy supports this assumption) and that the adsorbed layer thickness of polymer on CNCs was 10 nm.[25] Based on this analysis, 75% of the oil−water interface was stabilized by polymer-coated CNC particles and the remaining interface was saturated with adsorbed

polymer not associated with cellulose particles. The density of CNCs at the oil−water interface in our work was 1/4 of the value reported by Kalshnikova et al.[9,13] for emulsions prepared without water-soluble polymers. This combination of polymer-coated CNCs and free polymer as emulsifiers is sufficient to cover the entire emulsion droplet surface area and long-term stability is attributed to the physical/steric and kinetic stability of emulsifiers at the interface.

When emulsions are prepared, the order in which components are mixed matters. In all of the data presented above, CNCs and polymers were mixed prior to oil addition and emulsification. Alternatively, we prepared CNC-only stabilized emulsions followed by the addition of MC or HEC. When polymer was added in the second step, extensive coalescence and de-emulsification was observed (see the Supporting Information, Figure S8). Although CNC emulsion breaking is not the focus of this work, we beleive that adding adsorbing polymers after the emulsion is made, leads to fluctuation and reorganization of CNCs at the oil−water interface. Likewise, mixing all three components, HEC, MC, and CNCs, together before emulsification led to an abrupt viscosity increase in the suspension and emulsions could not be produced through sonication. For CNC-polymer emulsions where CNCs and polymers were mixed before emulsification, the surface of CNCs has been saturated with adsorbed polymers and therefore interaction between CNCs and polymers added later is blocked. This leads to emulsion stability upon addition of excess polymers and allows for the production of emulsion gels.

The emulsion gels studied here are straightforward to prepare (simple sonication plus heating) and contain only biobased polymers and particles; this is in contrast to many reports in the literature that require high-pressure treatment, acidification, and enzymatic cross-linking to induce gelation.[39,40] Specifically, CNCs and HEC/MC were mixed, the emulsion was then created, and additional MC was added to a concentration of about 1 wt % polymer. These emulsions at room temperature are more viscous than without the added polymer and turn to viscoelastic gels at higher temperatures (Figure 7b). Though a similar heating method has been used to prepare protein-based emulsion gels in food industry, higher concentrations (>6 wt %) of protein are normally required.[26] In addition, these heat-set protein-based emulsion gels involve protein denaturation at high temperatures and therefore are irreversible. By contrast, the gelation transition in our work is reversible and interestingly, the CNC-HEC+MC emulsion gels subjected to multiple cycles of heating and cooling did not display any noticeable change of emulsion droplet morphology (see the Supporting Information, Figure S7). As discussed, this thermoresponsive behavior of MC has been well-studied previously[34−36] and was easily exploited in this work to create temperature sensitive emulsion gels which we believe is an industrially relevant route to produce a new range of sustainable CNC-based products.

CONCLUSIONS

The combination of cellulose nanocrystals and adsorbing water-soluble cellulose derivatives has led to a synergistic stabilization of dodecane-water emulsions and emulsion gels. This work demonstrates the ability to carefully tailor emulsion properties with green emulsifiers and highlights the complexities of preparing multicomponent formulations. The major findings include: (1) A simple method to prepare stable emulsions with

tunable droplet size by using CNCs and water-soluble polymers. The emulsion stability was substantially enhanced when CNCs were first mixed with surface active, cellulose-adsorbing polymers (HEC or MC) followed by oil addition and emulsification. CNC–HEC and CNC–MC emulsions are more stable than emulsions stabilized by CNCs or polymers alone. (2) HEC and MC preferentially adsorb to CNCs over the oil–water interface; thus, to obtain the most stable emulsions, there must be sufficient polymer to saturate the CNC surfaces. The combination at the oil–water interface of polymer-coated CNCs (Pickering stabilization) and free polymer (high surface activity and fast interfacial partitioning) is responsible for the long-term emulsion stability observed in this work. (3) The sequence of mixing different emulsion components is critical. Only by first saturating CNCs with adsorbing HEC or MC are the most stable emulsion achieved. (4) Robust and reversible thermogelation was obtained for CNC–HEC emulsions upon addition of excess MC (a thermosensitive polymer). Multiple cycles of heating and cooling does not induce any noticeable emulsion coalescence, indicating the ultrastable nature of the emulsions gels.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Transmission electron microscopy (TEM) of CNCs, quartz crystal microbalance with dissipation monitoring (QCM-D) data for MC adsorption to cellulose, Malvern Mastersizer measurements of emulsions stabilized by polymers alone (without CNCs), photographs showing long-term emulsion stability, UV/visible spectroscopy data of fluorescently labeled emulsifiers, confocal laser scanning microscopy (CLSM) images of CNC–HEC+MC emulsions subjected to heat-treatment, CLSM of CNC stabilized emulsions with polymers added after emulsion formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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