Colloidal Behavior of Cellulose Nanocrystals Grafted with Poly(2-alkyl-2-oxazoline)

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Supporting Information

ABSTRACT: Polymer grafting onto cellulose nanocrystals (CNCs) has been used as a tool to improve CNC dispersion in nonpolar solvents or polymeric matrixes. The grafting of flexible polymer chains onto rigid particle surfaces leads to significant modifications in colloidal behavior. Here, poly(2-alkyl-2-oxazoline)s of well-defined molar mass and narrow molar mass distribution were synthesized by cationic ring-opening polymerization and grafted onto CNC surfaces, where the coupling reaction was favored when partially hydrolyzed polymers were used (reaching 64% reaction yield). The particles grafted with polymer chains could be redispersed in water after freeze-drying, producing stable dispersions, and they were not cell-toxic up to 10 wt % aqueous dispersion. Colloidal stability, nanostructure organization, and rheological behavior of grafted CNC and CNC-grafted CNC mixtures were evaluated. The rheological behavior of grafted nanoparticles, meanwhile, showed new features when compared to original CNC dispersions. Aqueous CNC dispersions showed a liquid crystal nematic organization and rheological behavior characteristic of true gel (at 5 wt %) prior to drying. On the other hand, nanoparticle dispersions behaved as weak gels upon the addition of 10 wt % of CNC-g-(PEtO_x95-Ei_5) under the same conditions. Dispersions of CNC-g-P(PEtO_x-s-Ei) particles obtained by redispersion of freeze-dried particles behaved as a fluid, without the presence of the nematic organization. Through oscillatory rheology and time-domain NMR results, it can be concluded that polymer-water interactions are dominant over CNC-water interactions, being responsible for CNC nematic phase disruption. By introducing polymer chains, the introduction of isotropic character modifies water organization, changing the flow behavior of CNC-grafted with poly(oxazoline)s.

INTRODUCTION

Surface modification of cellulose nanoparticles by polymer grafting is responsible for the increase in the use of cellulose nanocrystals (CNCs) and cellulose nanofibers for numerous applications.1-3 Due to the high number of hydroxyl groups on the surface of cellulose particles, several routes can be adopted to improve reaction efficiency and dispersion stability, which avoids aggregation of decorated nanoparticles.4

Classically, the grafting of hydrophobic polymers onto CNC surfaces has been used to enable their dispersion in nonpolar solvents, as well in polymeric matrices.5 On the other hand, hydrophilic polymers (especially responsive polymers) are grafted onto CNC surfaces for biomedical application purposes, imparting smart properties to the nanocrystals.5,6

Independent of the nature of the polymer or the CNC modification pathway, charged groups and tethered polymers contribute to the stability of aqueous CNC dispersions, reducing aggregation tendency.7-9 Surface modification can be quite advantageous; however, it does not change the general consensus of avoiding CNC drying whenever possible. Such a precaution is related to the aggregation of cellulose nanoparticles when water is removed from their interface.7,8

Both particle stabilization and the drying process affect the rheological behavior of these CNC-based materials, which is of extreme importance from the point of view of characterization and processing. Moreover, modifying CNCs by introducing polymer chains may impart relevant changes in particle organization in dispersion, and knowledge of the rheological behavior could be essential in revealing the dispersion of micro- and nanostructures.

Poly(2-oxazoline) (POx)-functionalized CNCs have shown promise in the design of CNC-based drug delivery systems, as shown by Hou and collaborators,10 who used POx as a polymer brush on CNC surfaces for photothermal cancer

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therapy. In this work, well-defined chains of poly(2-alkyl-oxazoline)s were grafted onto CNCs with the main objective of forming responsive hydrogels for biomedical applications. However, during redispersion of the modified CNCs in water, some questions were raised about their colloidal stability, such as their rheological behavior resulting from the structural organization of the particles and the micro- and nanostructural changes that the drying process brought to the system. Here, a grafted CNC is apparently well redispersed in water; however, small angle X-ray scattering (SAXS) and rheological measurements show that these dispersions did not present the properties they had before drying. The interaction between the poly(2-alkyl-oxazoline)s and water could be, in this case, the main explanation for this behavior, as discussed throughout the paper.

RESULTS AND DISCUSSION

2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO)-Oxidized CNCs. The CNC surface modification by TEMPO-mediated oxidation was completely characterized with reference to physicochemical properties to guarantee the inner characteristics of the pristine crystals. This procedure ensured a degree of oxidation (DO) of 0.05 (mol/mol of anhydroglucose unit), achieved by calculating the ratio of the intensity of the carbonyl absorption band (1736 cm$^{-1}$) to that of the strongest band at 1050 cm$^{-1}$, intrinsic to the cellulose backbone (Figure S1A). Similar results were obtained by conductometry (Figure S1B and Table S1). In addition, due to the presence of carboxylic groups on the surface of the oxidized CNCs, a ζ potential of −71.6 mV was observed in aqueous dispersions, higher than the values displayed by CNCs before TEMPO-mediated oxidation, of about −63.9 mV (Table S1).

The surface modification did not have any influence on the temperature of the maximum degradation rate. However, a slight decrease in the initial thermal degradation could be observed for the oxidized CNCs (Table S1), as already reported by other research groups.\textsuperscript{11,12} Pristine CNCs are highly crystalline and have been used as reinforcing agents for material in cellulose\textsuperscript{13} and herein, the CNCs showed a CI value of 83%, consistent with the values commonly presented in the literature.\textsuperscript{15} After TEMPO-mediated oxidation, a slight decrease in crystallinity to 76% was observed (Table S1). However, this did not compromise the general integrity of the crystalline domains, maintaining the CNCs’ high crystallinity to act as chemically modified reinforcing agents.

The physical integrity of the oxidized CNCs was confirmed by atomic force microscopy (AFM) and transmission electron microscopy (TEM) (Figure S4), as the aspect ratio (L/d) is an important characteristic, having an influence on several parameters such as the percolation threshold and also intrinsic mechanical properties.\textsuperscript{15} The micrographs shown in Figure S4 confirm that TEMPO-mediated oxidation preserves the integrity and shape of the CNCs by introducing carboxylate groups at their surface. Further information on the characterization results is given in the Supporting Information (Figures S1–S4 and Table S1).

Synthesis of Poly(2-alkyl-2-oxazoline). The poly(2-alkyl-2-oxazoline)s were synthesized by cationic ring-opening polymerization\textsuperscript{16,17} (Figure 1) and showed a narrow molar mass distribution (D < 1.2), determined by gel permeation chromatography (GPC) in chloroform (Table S2). More details of polymer synthesis and characterization are presented in the Supporting Information. GPC and UV analysis (Figure S5) results indicated the occurrence of transfer reactions as described by Warakomski et al.,\textsuperscript{18} which are responsible for polymer branching. Poly(2-alkyl-2-oxazoline) branching is not desirable because it imparts some changes to the polymer thermal properties and to the behavior of aqueous solutions (lower critical solution temperature, LCST). According to Flory–Fox,\textsuperscript{19} the glass transition temperature (T$\text{g}$) of a polymer increases with its molar mass. The poly(2-ethyl-2-oxazoline) (PEtOx) synthesized in this work, however, do not follow the Flory–Fox model. As branching takes place, the free polymer volume increases, contributing to decreased T$\text{g}$ (Figure S6). LCST, determined as the inflection point of the curve obtained by high sensitivity differential scanning calorimetry (HSDSC) (Figure S7), decreases by 5 °C for branched polymers with a higher molar mass (Table S2), probably due to the increase in the hydrophobic interactions between the polymer chains.

As uncontrolled branching imparts some changes in physicochemical properties, linear homopolymers (PEtOx\textsubscript{106}, Table S2) were the choice for partial side chain hydrolysis with diluted HCl solution\textsuperscript{20} to obtain poly(2-ethyl-2-oxazoline-stat-ethylene imine) (P(PEtOx-stat-Ei)) copolymers. The degree of hydrolysis was determined by $^1$H NMR (Figures S8 and S9 for PEtOx\textsubscript{106} and P(PEtOx-stat-Ei)—Supporting Information). Poly(ethyleneimine) synthesis is facilitated via POx hydrolysis; however, this can result in cytotoxicity and nonspecific interactions between polymer and cellular or noncellular components when in vivo application is desired.\textsuperscript{21} To verify

![Figure 1](synthesis_of_cnc.png)
the possible toxicity of these materials, P(EtOx-s-Ei) was evaluated regarding its in vitro cytotoxicity against fibroblast cell line NIH-3T3 using the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H]tetrazolium salt (MTS) assay. Results suggest that the polymers obtained after partial hydrolysis were safe up to 10 wt % in solution, with no significant differences in the number of viable cells compared to the control group (Figure S10). Furthermore, 5 or 10% hydrolysis did not show any influence on toxicity, enabling the application of these materials in biomedical studies.

**POx Grafting onto CNC Surfaces.** Well-defined POx chains were grafted onto the CNC surface by the peptidic coupling reaction (Figure 1). The Fourier transform infrared (FTIR) spectrum of freeze-dried CNC-g-POx showed two new features (Figure S11A): first, the displacement of the absorbed water band to 1627 cm$^{-1}$ due to the overlap with a new band of the amide II groups$^{22,23}$ and second, a relative decrease in the C=O band (1736 cm$^{-1}$) of the oxidized CNCs, which further supports the coupling reaction between the primary amines of POx and the carboxylic acids on the CNC surface. Covalent linkages between CNC and POx were further confirmed by the ninhydrin qualitative test.$^{24}$ The ninhydrin test is widely used for amino acid determination analysis through the oxidation of primary amines. Nonattached POx in solution with a primary amine as a terminal functional group provides a purple-colored solution, due to the Ruhemann’s purple complex formed from the condensation reaction between amine and ninhydrin.

Functionalized CNC dispersions did not show color changes after ninhydrin addition, unlike POx solution, which presented the characteristic purple color indicative of a positive reaction to primary amine (Figure S11).

To further confirm the coupling reaction, X-ray photoelectron spectroscopy (XPS) analysis was performed, as this technique is considered one of the most sensitive tools to investigate surface modification of CNCS.$^{25}$ Since XPS is a highly sensitive technique for atomic quantification in the solid state, it is suitable for obtaining quantitative information about the surface constituents and their chemical environment when the number of grafted polymer chains is too low.$^{26}$ The survey spectra of oxidized CNC and CNC functionalized with POx (Figure 2A,B) showed peaks at 288 and 533 eV, corresponding to carbon (C 1s) and oxygen (O 1s), respectively. After the coupling reaction, surface modification was confirmed by the appearance of a peak at 401 eV, which corresponds to nitrogen (N 1s), proving that there is some POx on the CNC surface. The N 1s XPS peak could be deconvoluted into three N peaks (Figure 2C): N1 at 401.8 eV, corresponding to the tertiary amide of the polymer lateral chain; N2 at 400 eV, corresponding to the secondary amide resulting from the coupling reaction between the carboxylic acid of the oxidized CNC and the primary amine at the end of the POx chain; and finally, N3 at 402.5 eV, attributed to the secondary amine from ethylenediamine used as the termination agent. The C 1s XPS peak can also be deconvoluted, as presented in Figure S12, showing an increase in the atomic concentration of C1 in CNC-g-POx compared to unmodified CNC, corresponding to the C−C/C−H linkages of the polymer grafted onto the CNC surface.

XPS analyses were performed on CNCS functionalized by P(EtOx-s-Ei) and, in this case, a higher nitrogen contribution was observed (Figure 2D). Indeed, the yield of the grafting reactions, calculated gravimetrically, was significantly higher when partially hydrolyzed polymer chains were used. Coupling
reaction efficiency was 12% for PEtOx grafting and between 58 and 64% when P(2(EtOx-s-Ei)) was grafted onto CNCs (the ninhydrin test proved no free polymer). The higher hydrophilicity of partially hydrolyzed polymers and also the higher availability of functional amine groups could be the keys to better reaction yields.

The decrease in $\zeta$-potential values from $-71.6$ to $-26.7$ mV is also indicative of the coupling reaction, and results from the presence of PEtOx chains on the CNC surface; for example, where negatively charged carboxylic groups are replaced by neutral polymer chains.

The grafted CNCs were analyzed by TEM and atomic force microscopy-infrared (AFM-IR). Although the pristine CNCs and TEMPO-oxidized CNCs presented definite edges (Figure 3A), as shown by the TEM images, the edges of grafted CNCs were less defined (Figure 3B) and presented a tendency to aggregate under drying (polymer-coated particles were well-dispersed in solution, as illustrated by the cryo-TEM image in Figure 3C). AFM-IR micrographs suggest that this low definition may be due to the coating of the CNCs by the grafted polymers. Figure 3D illustrates CNC coated by POx chains as flexible layers. With the IR laser adjusted to 1650 cm$^{-1}$, it was possible to map amide groups from POx lateral chains (Figure 3D); on the other hand, pristine CNCs did not show amide bands in the analyzed range (Figure S13, Supporting Information). Also, the presence of amide groups was only detected over or around the crystals; no signals were detected in the background. IR spectra collected in other parts of the image did not show bands characteristic of amide, as verified by the control image of PEtOx (Figure S14).

**Thermal Properties of CNC-g-POx.** Thermal properties such as degradation and glass transition temperatures can be affected by polymer grafting onto the CNC surface. CNCs used in this work showed thermal degradation with a maximum rate of mass loss at 302 °C (Table S2), while PEtOx degraded with a maximum rate at 411–426 °C (Table S1). Grafted POx modified the thermal profile of these materials. Thermal degradation of polymers and crystals occurs through different mechanisms and at different temperature ranges (Figure S15). One observed a reduction in the temperature of the maximum degradation rate of POx from 421 to 385 °C when these polymers were grafted onto the crystal surface, probably induced by pyrolysis of the CNCs. At temperatures higher than 200 °C, cellulose is responsible for the formation of flammable volatile compounds, changing the thermal diffusion mechanism in the polymer matrix and accelerating the degradation of the grafted polymer.27

Differential scanning calorimetry (DSC) analyses, in turn, did not show any thermal event related to the presence of CNC in the temperature range of −50 to 150 °C. The predominance of strong hydrogen bonds in the cellulose structure does not allow any thermal transitions before thermal degradation. According to previous theoretical extrapolation studies, CNC glass transition and melting temperatures would be 250 and 450 °C, respectively.28 Here, the DSC curves of CNC-g-P(2(EtOx-s-Ei)) showed a thermal transition related to the glass transition temperature of around 70 °C (Figure S16), typical of hydrophilic poly(oxazoline)s.29 The difference between the glass transition temperature of grafted (~70 °C) and free polymer (~45 °C) is considered a measure of
polymer chain mobility. These DSC curves suggest a loss of POx mobility after grafting onto rigid rod-like CNC surfaces, resulting in an increase of 25 °C in the T_g value (Figure S16), because the higher the glass transition temperature, the lower the chain mobility. This is in agreement with other polymer-grafted CNC studies, which showed an increase in the glass transition temperature due to the restricted mobility of the polymer chains grafted onto the CNC surface.30–32

**In Vitro Cytotoxicity Assay.** Given the potential of responsive polymer-grafted CNCs in biomedical applications, the cytotoxicity of pristine and CNC-g-POx was assessed using the MTS assay on fibroblast cell line NIH-3T3 (Figure S17). The functionalization of the CNC surface by different molecules has been carried out by different groups lately; however, little attention has been paid to toxicity. To guarantee the safe use of CNC-based materials in biomedical applications, it is important to evaluate the toxicity of these nanomaterials. Apparently, no toxicity of CNCs was demonstrated upon dermal or oral administration.35 However, some aspects might affect the toxicity of CNCs, such as the particle size and morphology, the degree of crystallinity, colloidal stability and, in particular, surface chemistry. In this last case, cytotoxicity is strongly dependent on the nature of the tethered chains and the chemistry used to access this functionalization. Preliminary evaluations have demonstrated that PEGylated CNCs are nontoxic for human ovarian cancer cell line (HEYA8).36 Also, poly(2-aminoethylmethacrylate)-g-CNC toxicity was evaluated against mouse monocyte cells (J774A1) and human breast adenocarcinoma cells (MCF-7).35 Even if no cytotoxicity to J774A1 cells was observed, an unexpected increase in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction was observed, requiring further investigations and demonstrating that such evaluations must be performed carefully case by case.

In this work, the cell viability of fibroblasts at different CNC-g-POx concentrations (0.1, 1, and 10 wt %) was evaluated after 24 h of incubation. There were no statistical differences (p < 0.05) of cell viability between the control (without CNC addition) and CNC-g-P(EtOx95-Ei5) (Figure S17). The presence of pristine and oxidized CNCs was not evaluated in this work, given their well-known safety.33 The results indicated that even at the highest concentration (10 wt %) CNC-g-P(EtOx95-Ei5) appeared to maintain cell viability close to 100%. However, CNC-g-P(EtOx95-Ei10) at 10 wt % decreased cell proliferation significantly after 24 h of incubation.

These preliminary results showed that all modified CNCs were safe in concentrations up to 1 wt % under the conditions tested in this assay. In the case of CNC-g-P(EtOx95-Ei5), higher concentrations could also be tolerated by the cells (up to 10 wt %). It should be highlighted here that, even though CNCs are considered nontoxic and the polymer used for grafting had been tested and proved to be safe, the final product imparted some cytotoxicity. The possible source of toxicity is unclear at the moment. This open question will be investigated further in more detail.

**CNC-g-POx Particle Dispersion.** CNC particles are usually obtained by sulfuric acid hydrolysis. The presence of charged sulfate ester groups on crystallite surfaces results in negatively charged particles and stable aqueous dispersions at low ionic strengths, with unique colloidal properties.37 With drying, CNC particle–particle interactions are very strong and are dominated by hydrogen bonding and attractive van der Waals forces, rendering complete redispersion a challenge. Here, pristine CNC dispersion was not reached after drying, even after sonication, whereas CNC-g-P(EtOx95-Ei5) was completely dispersed in water without extra energy requirement. Dispersions of mixtures of CNC-g-P(EtOx95-Ei5) with different degrees of hydrolysis (5 and 10%) and different CNC mass ratios (0, 0.5, 1, 2, 4, and 10) were freeze-dried and redispersed in water at 0.01 wt % concentration. These dispersions and pristine CNC dispersions were analyzed by dynamic light scattering (DLS). Figure 4 illustrates the mean diameter and polydispersity index (PDI) of never-dried pristine CNCs and CNC-g-P(EtOx95-Ei5) particles redispersed in water as a function of the CNC/CNC-g-P(EtOx95-Ei5) mass ratio, determined by DLS.

Regardless of the degree of side chain hydrolysis of the grafted polymer, particle size (Figure 4A) and PDI (Figure 4B) of the redispersions resulted in similar behavior. Besides, CNC-g-P(EtOx95-Ei5) favored pristine CNC dispersion. The gradual addition of pristine CNCs to CNC-g-P(EtOx95-Ei5) (CNC/ CNC-g-P(EtOx95-Ei5), 1:1–10:1, w/w) before freeze-drying facilitated redispersion in water due to the steric effect of the polymer grafted onto the CNC surface. CNC-g-P(EtOx95-Ei5)s particles showed a hydrodynamic radius (R_h) around 350–400 nm after freeze-drying and redispersion in water (Figure 4A). With the addition of pristine CNCs, R_h decreased as its concentration ratio increased, reaching a constant value of around 150 nm, with a monomodal size distribution (Figure S18) and low PDI, close to the values reached in never-dried pristine CNCs (red closed circles in Figure 4). The monomodal particle size distribution (Figure S18) suggests that the reduction in PDI is not due to the presence of smaller particles (CNC), for which two particle populations should be observed. Therefore, we believe that the presence of pristine CNC particles behaves as a steric stabilizing agent, able to prevent the formation of dynamic aggregates due to polymer chain entanglement. Dispersed crystals in water, with no aggregates, were observed by cryogenic microscopy, represented by the image of a CNC-g-P(EtOx95-Ei5) dispersion (0.1 wt %) in Figure 3C.

Stable colloidal dispersions of modified CNCs were also obtained by grafting hydrophilic polymers such as Jeffamine (a statistical copolymer of ethylene oxide and propylene oxide) and poly(ethylene oxide) onto a CNC surface. However, the ability of redispersion of these particles after drying in aqueous
This ability to redisperse CNC particles after drying is relevant since many properties attributed to CNCs are based on their ability to assemble spontaneously into ordered structures. Called a nematic phase, such organization is reached above a critical concentration. For CNC particles, a phase transition occurs from an isotropic phase composed of particles distributed randomly to an anisotropic phase, in which the needle-like particles point in the same direction, characterizing a chiral nematic liquid crystalline phase. The critical volume fraction of particles for this transition depends on superﬁcial charges and the aspect ratio of the nanoparticles.\textsuperscript{41,42} Here, the nematic organization was observed through SAXS measurements for CNC dispersions at 5 wt % concentration as a broad peak centered at $q = 0.1 \text{ nm}^{-1}$ corresponding to a period of $\sim 63 \text{ nm}$. This primary scattering peak is related to the center-to-center separation distance between neighboring particles ($d = 2\pi/q_{\text{peak}}$; $q$ is the scattering vector).\textsuperscript{44,45} Then, the addition of CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s) (in a ratio of 10:1 CNC/CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s), w/w) into a CNC dispersion imparts some tenuous structural changes, however, maintaining the nematic phase (Figure 5). The shift of the scattering Bragg peak toward higher $q$ in the presence of polymer-grafted CNCs indicates a slight decrease in CNC interparticle distance to $\sim 57 \text{ nm}$. A secondary, less intense scattering peak ($q \sim 0.2 \text{ nm}^{-1}$) was also observed, which can be related to the formation of anisotropic two-dimensional CNC aggregates.\textsuperscript{46} CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s) was the grafted CNC of choice for further colloidal studies since no cytotoxicity was observed up to 10 wt % and its colloidal behavior in aqueous dispersion (size distribution, PDI) showed no signiﬁcant difference compared to CNC-g-P(\text{EtOx}_{90-s}\text{-Et}_{10}).

Although CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s) showed a satisfactory dispersion in water after freeze-drying, a CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s) dispersion at 5 wt % did not show structural nematic organization, being isotropic. Similarly, well-dispersed pristine CNCs with CNC-g-P(\text{EtOx}_{95-s}\text{-Et}_s) at a 10:1 ratio was isotropic at 5 wt % when the physical mixture was redispersed in water after drying. A visual consequence was apparent when the gel test was performed. Samples with the characteristic nematic organization did not flow with time, while isotropic dispersions behaved as a ﬂuid of low viscosity (Figure 5). Investigation of the gravity-driven ﬂowability of CNC dispersions was made at different concentrations by Liu et al.,\textsuperscript{45} whose work suggests that the gelation threshold of CNCs is around 6 wt %, corresponding to the concentration associated with structural organization dominated by the nematic phase. There is an undoubted correlation between gelation and the presence of a chiral nematic liquid crystalline phase.
phase. This should be an important point of study and characterization for future applications when redispersion of modified CNCs is required since grafted CNC organization and gelation properties are influenced by the presence of tethered polymers.

**Rheological Properties.** Rheological properties of aqueous dispersions of pristine CNCs and CNC-g-P(EtOx55-5-Ei5) were investigated in the semidilute regime (5 wt %), in which particles are interacting, resulting in self-assembly.55

Figure 6A presents the evolution of complex viscosity as a function of angular frequency for the pristine CNC dispersion, in which pristine CNCs are oriented in a nematic phase, as shown by SAXS (Figure 5). A decrease in the complex viscosity was observed with increasing angular frequency, characteristic of shear-thinning behavior and typical of CNC dispersions.19,56 The applied stress led progressively to the flowing of anisotropic domains as a result of particle orientation in the flow caused by the shear. As a consequence, the liquid crystal domains were broken up with a further increase in the frequency, causing a decrease in the drag force, reducing viscosity.

Oscillatory experiments were performed to understand the rheological behavior of the dispersions of pristine CNCs (rigid rod-like particles) and grafted CNCs (rigid fillers with soft polymer chains in a brush-like structure), prepared in different conditions.57 For pristine CNC dispersions, the magnitudes of $G'$ and $G''$ were almost independent of frequency (Figure 6A). According to dynamic experiments, storage modulus ($G'$) values were greater than loss modulus ($G''$) values over the whole range of frequencies evaluated, corresponding to elastic behavior.53 In addition, the ratio of $G'$ to $G''$ ($\tan \delta$) was less than 0.1 in the range of angular frequency studied (Figure S19A), meaning that the sample is a true gel.12,54

The addition of CNC-g-P(EtOx55-5-Ei5) to the pristine CNC dispersion (CNC/CNC-g-P(EtOx55-5-Ei5) = 10:1, total CNC concentration in water of 5 wt %), on the other hand, highlighted particular structural changes that were slightly apparent in SAXS measurements (Figure 6B). First, $G'$ at rest (0.01 Hz) showed a remarkable decrease of almost 4 order of magnitude compared to $G'$ of pristine CNC dispersions. This reduction was accompanied by a decrease in viscosity of 3 orders of magnitude. However, as the frequency increased to 0.03 Hz, a shear-thickening behavior was observed. This behavior is probably related to the coil-to-stretch transition experienced by the tethered polymer chains under oscillatory shearing, wherein the molecule unravels to a nearly fully extended state.55

Later, at the point where angular frequency reaches the maximum value of viscosity (0.03 Hz), a shear-thinning behavior was seen. This happens because the effect of polymer rigidity on the viscosity is no longer dominant over the shearing, so the alignment of the crystals in the flow direction is established, followed by breaking of the nematic organization.52

$\tan \delta$ values also showed important changes over the angular frequency range: the transition from liquid-like to solid-like behavior was observed at the crossover frequency, at which $\tan \delta \approx 1$ (Figure S19). Coincidently, this sol–gel transition occurred at the exact point at which the shear-thinning behavior began. It is worth mentioning that at low frequency the $\tan \delta$ value was 5.75, characteristic of a viscous liquid. When a higher frequency was applied $\tan \delta$ reached a value of 0.17, and the CNC dispersion in the presence of 10 wt % CNC-g-POx could be considered as a weak gel.54 Such low values reinforce the hypothesis that the nanoparticle self-assembly was not present in these samples.

A CNC dispersion obtained from a physical mixture of pristine CNCs and CNC-g-P(EtOx55-5-Ei5) followed by freeze-drying and redispersion in water (5 wt %) showed a significant reduction in complex viscosity, $G'$ and $G''$ moduli, of 5, 8, and 4 orders of magnitude, respectively. The CNC-g-P(EtOx55-5-Ei5) dispersion presented similar behavior (Figure S20).

Finally, one observed that the presence of pristine CNCs in the CNC-g-P(EtOx55-5-Ei5) dispersion had a slight effect of increasing viscosity. Both $G'$ and $G''$ values increased with angular frequency, and $G''$ remained higher or close to $G'$ over the whole angular frequency range investigated. Even at higher frequencies, these materials can be considered as liquid fluids, with no sol–gel transition, whose complex viscosity increases as the angular frequency increases. In this case, when the CNC nematic organization is not present, the polymer coil-to-stretch transition is predominant in the dispersion and is observed up to higher frequencies (blue and green circles in Figure S20).

**Gel Structure.** Despite CNC concentration remaining the same in all studied dispersions, different flow behaviors were observed, depending on the presence of polymer chains and the procedure of dispersion preparation (never-dried CNCs or dispersion after freeze-drying). It is known that CNC dispersions change progressively from an isotropic structure to a biphasic isotropic and liquid crystalline structure, to a liquid crystalline structure, and then to a gel-like structure with increasing concentration.56 A true gel-like flow behavior was observed for the pristine CNC dispersions prepared at 5 wt %; however, similar behavior is not verified for other dispersions.

Through time-domain NMR (TDNMR) analysis one can better understand the difference between these flow behaviors and predict the material network. One of the most common TDNMR applications is the characterization of sol–gel transitions and hydrogels where three-dimensional organization affects the magnetic relaxation of the water hydrogens.57 Water mobility is revealed depending on the spin–spin relaxation time ($T_2$), in which the component with the shortest relaxation time, in the range of $T_2 < 10$ ms, is due to the bound water. The second component, with signals in the range of 10–100 ms refers to immobilized water, while the signals with the longest relaxation time (100–1000 ms) are considered to be due to free water.58

Herein, the nematic liquid crystal organization remained stable with the addition of CNC-g-P(EtOx55-5-Ei5) as observed by SAXS; however, an increase in the relaxation time of the immobilized water (91 ms) compared with the pristine CNCs dispersion (79 ms) was observed by TDNMR, which indicates an increase in water mobility (Figure 7). The change of water mobility caused by the presence of POx chains influences flow properties, leading to a decay in viscosity and the rheological behavior of a weak gel at high angular frequencies.

When CNC/CNC-g-P(EtOx55-5-Ei5) (10:1) dispersion was subjected to freeze-drying, followed by redispersion in water at the same concentration, a substantial rheological alteration occurred (i.e., it flowed). In addition to the lack of nematic phase, one observed an even greater increase in the relaxation time related to the immobilized water (107 ms), which indicates higher water mobility compared to the never-dried dispersion (Figure 7). The dispersion obtained from pure CNC-g-P(EtOx55-5-Ei5) in turn, showed a similar flow behavior, with even lower viscosity. The spectrum resolved
by TDNMR analysis shows that the water present in this system was predominantly free water (705 ms, Figure 7). Moreover, a discrete signal around 10 ms indicates the presence of bound water, characteristic of water tightly attached to macromolecules. This last result suggests that water−polymer interactions became dominant and more important than those water−CNC and CNC−CNC interactions after freeze-drying and redispersion in CNC-g-P(EtOx95-s-Ei5) dispersion. Besides, in the case of pure CNC-g-P(EtOx95-s-Ei5) dispersion, a higher concentration of polymer was present since no pristine CNC was added, contributing to polymer−water interactions which favored the appearance of the bound water in the system.

To illustrate better the influence of particle interactions in the rheological behavior of CNC dispersions in the presence of polymer chains, Figure 8 shows a possible CNC−polymer network organization based on rheological behavior and water displacement, as discussed by Li et al.

Negatively charged CNCs with a large number of hydroxyl groups on the surface are able to interact with each other as well as with adjacent water molecules. Due to the rigidity of CNC particles, the network is composed of interactions between different crystals and crystal-immobilized water molecules (Figure 8A). The presence of flexible polymer chains, on the other hand, disrupts water organization. Physical entanglement of polymer chains occurs, together with interactions between polymer chains and water molecules. These interactions promote an increase in the mobility of immobilized water without changing the anisotropy of the dispersion (Figure 8B).

When freeze-dried material is redispersed in water, particle organization is controlled by polymer hydration and not by CNC electrostatic repulsion, which results in an isotropic dispersion with increased water mobility and interparticle distance increases, forming a less packed network (Figure 8C). This hypothesis is reinforced by the dispersion prepared only with CNC-g-P(EtOx95-s-Ei5) (Figure 8D), which contains the same CNC concentration, however, contains a higher concentration of P(EtOx95-s-Ei5). The presence of hydrophilic polymers surrounding CNC particles prevents water immobilization by the CNC network. In this case, water with a relatively higher degree of freedom is dominant in the system, accompanied by the presence of water bound to hydrophilic polymer chains.

**CONCLUSIONS**

Well-defined POxs were synthesized and successfully grafted onto CNC surfaces by a grafting-to approach. Hydrolysis of PETox favored grafting onto CNCs, increasing the reaction yield to 64%. CNC-g-POx in turn, showed some thermal property changes compared to starting materials. The polymer grafting onto CNCs caused a decrease in the temperature of the maximum degradation rate of POx and an increase in $T_g$ of
around 25 °C due to the reduction in the polymer chain flexibility.

CNC-g-POx dispersions are safe at low concentrations (0.1–1 wt %) according to the MTS assay performed on fibroblast cells. On the other hand, a significant reduction in cell proliferation was observed at higher concentrations of CNC-g-P(EtOx95-s-Ei5) (10 wt %), whereas no toxicity was observed for CNC-g-P(EtOx50-s-Ei5) under the same conditions. Dispersions of modified CNCs by polymer grafting are stable, with no aggregation and with particles presenting monomodal distribution. However, different flow behavior properties were observed. A gel test showed that a pristine CNC dispersion and a CNC/CNC-g-P(EtOx50-s-Ei5) (10:1) dispersion did not flow under the force of gravity, whereas dispersions prepared by redispersion of freeze-dried samples in water flowed under stress or gravitational field. SAXS measurements demonstrated that a nematic organization could be responsible for the gel-like behavior observed for pristine CNC and CNC/CNC-g-P(EtOx50-s-Ei5) (10:1) dispersions. Such an organization seems to be irreversibly broken during freezing.

Oscillatory rheology assays revealed the influence of polymer chains on flow behavior. Although a pristine CNC dispersion presented shear-thinning behavior and a true gels character, a weak gel behavior was observed after addition of CNC-g-P(EtOx50-s-Ei5). The presence of tethered polymer chains imparts rheological changes since shear-thickening behavior characteristic of polymers was observed with increasing angular frequency for this sample. TDNMR measurements, in turn, suggest that the mobility of water molecules in the presence of polymer chains is also responsible for the observed rheological behavior for modified CNCs. This highlights new features of CNC-based hydrogel structures that are usually not considered for this type of material. Water displacement was related to polymer–water and CNC–water interactions, revealing particle organization before and after freeze-drying of CNC-g-POX dispersions.

### EXPERIMENTAL SECTION

#### Materials

Cellulose nanocrystal dispersion (University of Maine, 13 wt %) was used after extensive dialysis against deionized water. Acetonitrile (Vetc, 99.9%), methyl p-toluene sulfonate (MeOTs) (Sigma-Aldrich, 97%), and 2-ethyl-2-oxazoline (Sigma-Aldrich, 99%) were stored over toluenesulfonate (MeOTs) (Sigma-Aldrich, 97%), and 2-ethyl-2-oxazoline (Sigma-Aldrich, 99%), diethyl ether (Synth, 98%), calcium hydride (Acros Organics, 93%), ethylenediamine (MeOTs) was added with a nitrogen-purged syringe, followed by the addition of 2-ethyl-2-oxazoline (10 mL). The flask was then heated at 120 °C for 1 h, the reaction was quenched by adding ethylene diamine (3 equiv) and stirred at room temperature overnight. The polymer was precipitated in diethyl ether and dried under vacuum.

Poly(2-ethyl-2-oxazoline) (2). In a typical polymerization procedure, acetonitrile (12 mL) was introduced into a dried Schlenk flask under vacuum. To this, depending on the degree of polymerization of the POx, a specific amount of the initiator (MeOTs) was added with a nitrogen-purged syringe, followed by the addition of 2-ethyl-2-oxazoline (10 mL). The flask was then heated at 120 °C for 1 h, the reaction was quenched by adding ethylene diamine (3 equiv) and stirred at room temperature overnight. The polymer was precipitated in diethyl ether and dried under vacuum.

Poly(2-ethyl-stat-ethylene imine) (3). Poly(2-ethyl-2-oxazoline) was dissolved in water in a concentration of 100 g L⁻¹, 6 M HCl was added (1:1, water/HCl, v/v) and heated to 100 °C under reflux. After 2 h, the solution was left to equilibrate at room temperature and a solution of 2.5 M NaOH was slowly added until pH 8. Dialysis was performed against deionized water for 3 days and grafting of poly(2-ethyl-2-oxazoline-stat-ethylene imine) copolymers were obtained after freeze-drying as a white powder.

CNC-g-POX (4). Grafting of amine-terminated poly(2-alkyl-2-oxazoline)s (POx) was achieved through peptidic coupling reaction by dissolving the pure polymer to a 1 wt % carboxylated CNC (1:1, primary amine/carboxylic group) at room temperature. EDC (5 equiv) and NHS (5 equiv) were added to the dispersion and the reaction was run for 24 h. Grafted CNC was purified by precipitation in 0.5 M NaCl solution to remove reagents and nongrafted POx, followed by centrifugation (10 000 rpm/10 min), dialysis against deionized water, and freeze-drying. Grafting yield was determined by gravimetry after dialysis and negative result of ninhydrin qualitative test.

#### Chemical Characterization

The carboxyl content of oxidized CNCs was determined by FTIR for freeze-dried CNC dispersions. FTIR analyses were performed in an ATR mode on Agilent Cary 630 equipment between 400 and 4000 cm⁻¹, with a 4 cm⁻¹ resolution. For conductometric analysis, the CNCs (70 mg) were dispersed in 30 mL of deionized water and 0.1 M HCl was added (1 mL). After 5 min of dispersion in an ultrasonic bath, the dispersions were titrated with 0.01 M NaOH. Typical titration curves (Figure S1, Supporting Information) showed the presence of strong acid due to the excess of HCl and weak acid corresponding to the carboxyl content, allowing the determination of the degree of oxidation (DO), which was calculated as proposed by da Silva Perez et al.¹⁰

¹H and ¹³C NMR solid state spectra were obtained on a Bruker 400 spectrometer operating at 10 kHz. The grafting of...
POx onto CNC surfaces was evaluated by XPS for freeze-dried CNC-g-POx samples using a Thermo K-Alpha XPS spectrometer (Thermo Scientific, Inc.) with monochromatic Al Kα X-ray radiation (1486 eV). Spectra were collected at three locations and elemental compositions were determined from low-resolution survey measurements with 200 eV pass energy and 1 eV steps, whereas high-resolution spectra were collected with 50 eV pass energy and 0.01 eV steps. Molar mass distribution and polydispersity of the polymers were determined by GPC, performed on a Viscotek GPCmax VE2001 instrument equipped with three Shodex columns (K-802, K-803, and K-804) and Viscotek VE3580 refractive index and Viscotek UV 2500 detectors at 40 °C using chloroform as the eluent at a flow rate of 1 mL min⁻¹ and polystyrene standards for calibration.

**Thermal Characterization.** The thermal behavior of polymers and CNCs was characterized by thermogravimetric analysis (TGA) using TA Instruments 2150 equipment under air at 100 mL min⁻¹. Analyses were performed from room temperature to 900 °C with a heating rate of 10 °C min⁻¹, after isothermal at 80 °C until constant mass. Polymer phase transition analyses were performed in a DSC Q2000TA instrument (New Castle, DE), using the following program: (i) heating from 20 to 150 °C; (ii) isotherm for 2 min; (iii) cooling from 150 to −50 °C; (iv) isotherm for 10 min; (v) heating from −50 to 150 °C. The scans were carried out at a rate of 20 °C min⁻¹ and the analyses were conducted under an argon atmosphere (50 mL min⁻¹ flow rate). DSC curves were normalized by the mass of the sample used in each analysis. The lower critical solution temperature of POx aqueous solutions was determined by HSDSC, performed on a MicroCal VP-DSC (Northampton, MA) calorimeter. Aqueous polymeric solutions (20 mg mL⁻¹) were added to a 0.5 mL cell and scanned from 10 to 100 °C at a heating rate of 1 °C min⁻¹. Data obtained were treated with Origin 7.0 software.

**X-ray Diffraction (XRD).** XRD patterns of CNCs and modified CNC powders were recorded in the range of 2θ = 5−50° at a scan speed of 2° min⁻¹ in steps of 0.02° with a Shimadzu XRD 7000 X-ray diffractometer. Cu Kα radiation (λ = 0.1542 nm) was used with a generator voltage of 40 kV and a current of 30 mA. The CI was determined by the peak height method, using the following equation

\[ CI = \left( I_1 - I_2 \right) / I_2 \]

where \( I_1 \) is the intensity of the peak at the minimum (2θ = 18.2) and \( I_2 \) is the intensity of the peak associated with the crystalline region of CNCs (2θ = 22.7).borough

**Atomic Force Microscopy (AFM).** AFM characterization was performed using a NanoSurf Flex AFM microscope, under a controlled atmosphere of <30% relative humidity by flowing \( N_2 \) gas into the chamber. Atomic force microscopy with infrared spectroscopy (AFM-IR), in turn, was performed with a NanoIR2s Anasys microscope from an infrared radiation source (Quantum Cascade Lasers—Daylight) set up to follow the carbonyl (C=O) group from amide (1650 cm⁻¹). A drop (10 μL) of a diluted aqueous CNC dispersion (0.01 wt %) was deposited on a mica substrate and dried overnight. The crystals were imaged in the intermittent contact mode using a silicon cantilever having a nominal tip radius of <10 nm.

**Transmission Electron Microscopy (TEM).** TEM images were recorded in low-dose conditions using a TAALOS F200C (Thermo Fischer Scientific) microscope working at 80 kV equipped with a Ceta 16 M 4 k × 4 k pixel CMOS camera (Thermo Fischer Scientific). TEM samples were prepared by the deposition of CNC-g-POx dispersions (0.01 wt %) on a carbon 400 mesh copper grid (Ted Pella Inc.) and removing the excess after 1 min, followed by staining with a 1% uranyl acetate solution for 30 s.

Cryo-TEM images were recorded in low-dose conditions, with a defocus range of 1−3 μm, using a TALOS F200C (Thermo Fischer Scientific) microscope working at 80 kV equipped with a Ceta 16 M 4 k × 4 k pixel CMOS camera (Thermo Fischer Scientific). Samples were prepared by deposition of CNC-g-POx dispersions (0.1 wt %) onto a Lacey Carbon 300 mesh copper grid (Ted Pella Inc.) in a controlled environment vitrification system (Vitrobot Mark IV, Thermo Fischer Scientific—formerly FEI) at controlled temperature (22 °C) in 100% humidity, blot force of 5 and blot time of 2.5 s.

For both sample preparations, grids were previously subjected to a glow discharge treatment using an easiGlow discharge system (Pelco) with 15 mA negative current for 10 s in air atmosphere to make them hydrophilic. Sample preparation and data acquisition were performed at the Electron Microscopy Laboratory/Brazilian Nanotechnology National Laboratory (LNNano).

**Cell Viability.** MTS assay was performed to evaluate the cytotoxicity of partially hydrolyzed polymers (POx) and CNC-g-POx toward fibroblast cell line NIH-3T3. Fibroblasts were seeded into 96-well plates at a density of 10⁴ cells per well. The cells in monolayers were treated with Dulbecco’s modified Eagle’s medium (supplemented with 10% fetal bovine serum, penicillin (100 U mL⁻¹), streptomycin (100 μg mL⁻¹) in a humidified 5% CO₂ atmosphere at 37 °C) with increasing concentration of POx and CNC-g-POx (1, 5, 10%) for 24 h. Wells with serum-free medium were used as a negative control and with dimethyl suloxide aqueous solution (50%) as a positive control. For the MTS assay, the CellTiter 96 AQueous One Solution Cell Proliferation Assay kit was used following the manufacturer’s instructions. Briefly, 20 μL of the MTS reagent was added into each well and the cells were incubated at 37 °C for 2 h. The absorbance was measured at 490 nm with a PerkinElmer microplate reader. All experiments were repeated twice, in triplicate. CNC-g-POx and its concentration effects were tested using analysis of variance and significant differences between the averages were determined by the Tukey test at 95% probability with OriginPro 8.0 software.

**Dynamic Light Scattering (DLS) and ζ Potential.** DLS and ζ potential measurements were carried out with Zetasizer Nano Series (NANO-ZS) equipment from Malvern Instruments for diluted dispersions (0.01 wt %) of CNC, CNC-g-POx, and physical mixtures of pristine and modified CNCs prepared by freeze-drying and redispersion in water at 0.1 wt % in different mass ratios (CNC/CNC-g-POx, 0.1:1, 1:1, 2:1, 4:1, 10:1, w/w).

**Small Angle X-ray Scattering (SAXS).** CNC structural organization was studied by SAXS from dispersions at a concentration of 5 wt %. The measurements were performed using the SAXS beamline at the Brazilian Synchrotron Light Source (LNLS), in Campinas, Brazil. The wavelength used was 1.548 Å and the sample-to-detector distance was 3 m. The measurements were taken at 25 °C and the sample holder was a mica window cell with a spacing of 1 mm. Fit2D software was used to subtract background and solvent scattering and the results are expressed in terms of X-ray scattered intensity, \( I(q) \), versus the scattering vector \( q \).
Rheology. The rheological behavior of CNC dispersions was characterized in oscillatory mode (small amplitude oscillatory shear, SAOS) using a Thermo Scientific HAAKE Mars III rheometer equipped with plate–plate geometry (35 mm diameter and 1 mm gap). The oscillatory experiments were carried out within the linear viscoelastic range as determined by strain sweep measurements. The storage modulus ($G'$) and loss modulus ($G''$) were obtained from dynamic stress sweep spectra at 25 °C, in the angular frequency range of 0.01−100 Hz.

Time-Domain NMR (TDNMR). TDNMR measurements were performed on a Bruker Minispec mq20 NMR analyzer (Bruker Company) at a proton resonance frequency of 20 MHz. Samples were placed in an 8 mm diameter glass tube and inserted in the NMR probe, and the temperature at 30.8 ± 0.2 °C was stabilized for 15 min. The spin–spin relaxation time, $T_2$, was measured using the Carr– Purcell–Meiboom–Gill (CPMG) sequence, with 90 and 180 proton pulses of 8.4 and 16.7 ms, respectively, and echo time of 160 ms. The data were obtained in triplicate, in which 30 000 echoes were acquired with 16 scans with the time between subsequent scans of 15 s. The fitting of the CPMG decay curves was performed using the multi-exponential fit.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01269.

FTIR and $^{13}$C NMR spectra, XRD diffraction patterns, AFM and TEM images for pristine and oxidized CNCs; conductometric titration curves for oxidized CNC; main characteristics for pristine and oxidized CNCs; illustrative image of polymer color changes as a function of time reaction; GPC chromatograms for PEtOx; maximum of absorbance values of PEtOx aqueous solution; mechanism of chain transfer reaction; DSC second heating scan for PEtOx; HSDSC first heating for PEtOx$_{106}$ aqueous solution; $^1$H NMR spectra for PEtOx and P(EtOx-$s$-Et$i$); cell viability determined by the MTS assay for P(EtOx-$s$-Et$i$); FTIR spectra for TEMPO-oxidized CNCs and after PEtOx grafting onto the CNC surface; ninhydrin test; C 1s XPS binding energy for TEMPO-oxidized CNCs and CNC-PEtOx; AFM-IR for pristine CNCs and PEtOx; TGA and dTGA curves for P(EtOx$_{95}$-$s$-Et$i$)$_{100}$, CNC-PEtOx$_{95}$-$s$-Et$i$)$_{100}$ and pristine CNC; DSC second heating scan for POx and CNC-POx; cell viability determined by MTS assay for CNC-PEtOx$_{95}$-$s$-Et$i$; size distribution and tan δ values for never-dried pristine CNCs and CNC + CNC-PEtOx$_{95}$-$s$-Et$i$; complex viscosity, storage ($G'$) and loss ($G''$) moduli as a function of oscillatory frequency for pristine CNC-based dispersions (PDF)

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This manuscript was written with contributions from all authors. All authors have confirmed the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wohlfahner, S.; Delepiere, G.; LABELT, M.; Morandi, G.; Thielemans, W.; Weder, C.; Zappe, J. O. Grafitando Polymersf rom Cellulose Nanocrystals: Synthesis, Properties, and Applications. Macromolecules 2018, 51, 6157–6189.

(2) Habbibi, Y. Key advances in the chemical modification of nanocelluloses. Chem. Soc. Rev. 2014, 43, 1519–1542.

(3) Kedzior, S. A.; Kiriakou, M.; Niinivaara, E.; Dubé, M. A.; Fraschini, C.; Berry, R. M.; Cranston, E. D. Incorporating Cellulose Nanocrystals into the Core of Polymer Latex Particles via Polymer Grafting. ACS Macro Lett. 2018, 7, 990–996.

(4) Kargarzadeh, H.; Huang, J.; Lin, N.; Ahmad, I.; Mariano, M.; Dufresne, A.; Thomas, S.; Galeski, A. Recent developments in nanocellulose-based biodegradable polymers, thermoplastic polymers, and porous nanocomposites. Prog. Polym. Sci. 2018, 87, 197–227.

(5) Kan, K. H. M.; Li, J.; Wijesekera, K.; Cranston, E. D. Polymer-Grafted Cellulose Nanocrystals as pH-Responsive Reversible Flocculants. Biomacromolecules 2013, 14, 3130–3139.

(6) Brinatti, C.; Ahklighi, S. P.; Pires-Oliveira, R.; Bernardinelli, O. D.; Berry, R. M.; Tam, K. C.; Loh, W. Controlled coagulation and redispersion of thermo-responsive poly di(ethylene oxide) methyl ether methacrylate grafted cellulose nanocrystals. J. Colloid Interface Sci. 2019, 538, 51–61.

(7) Khoshkava, V.; Kamal, M. R. Effect of drying conditions on cellulose nanocrystal (CNC) agglomerate porosity and dispersibility in polymer nanocomposites. Powder Technol. 2014, 261, 288–298.

(8) Han, J.; Zhou, C.; Wu, Y.; Liu, F.; Wu, Q. Self-assembling behavior of cellulose nanoparticles during freeze-drying: Effect of suspension concentration, particle size, crystal structure, and surface charge. Biomacromolecules 2013, 14, 1529–1540.

(9) Beck, S.; Bouchard, J.; Berry, R. Dispensibility in water of dried nanocrystalline cellulose. Biomacromolecules 2012, 13, 1486–1494.

(10) Hou, L.; Fang, J.; Wang, W.; Xie, Z.; Dong, D.; Zhang, N. Indocyanine green-functionalized bottle brushes of poly(2-oxazoline) on cellulose nanocrystals for photothermal cancer therapy. J. Mater. Chem. B 2017, 5, 3348–3354.

(11) Fraschini, C.; Chauve, G.; Bouchard, J. TEMPO-mediated surface oxidation of cellulose nanocrystals (CNCs). Cellulose 2017, 24, 2775–2790.

(12) Mariano, M.; Kissi, N. E.; Dufresne, A. Cellulose nanomaterials: size and surface influence on the thermal and rheological behavior. Polymers 2018, 28, 93–102.

(13) Park, S.; Baker, J. O.; Himmel, M. E.; Park, P. A.; Johnson, D. K. Cellulose crystallinity index: measurement techniques and their
impact on interpreting cellulose performance. Biotechnol. Biofuels 2010, 3, No. 10.

(14) Kargarzadeh, H.; Mariano, M.; Gopakumar, D.; Ahmad, I.; Thomas, S.; Dufresne, A.; Huang, J.; Lin, N. Advances in cellulose nanomaterials. Cellulose 2018, 25, 2151–2189.

(15) Bras, J.; Viet, D.; Bruzese, C.; Dufresne, A. Correlation between stiffness of sheets prepared from cellulose whiskers and nanoparticles dimensions. Carbohydr. Polym. 2011, 84, 211–215.

(16) Hoogenboom, R. Poly(2-oxazoline): Alive and Kicking. Macromol. Chem. Phys. 2007, 208, 18–25.

(17) Legros, C. Engineering of Poly(2-oxazoline)s for Potential Use in Biomedical Applications: Polymers. Université de Bordeaux, 2014.

(18) Warakomski, J. M.; Thill, B. P. Evidence for long chain branching in polyethyloxazoline. J. Polym. Sci., Part A: Polym. Chem. 1990, 28, 3551–3563.

(19) Fox, T. G.; Flory, P. J. Second-order transition temperatures and related properties of polystyrene. I. Influence of molecular weight. J. Appl. Phys. 1950, 21, 581–591.

(20) Legros, C.; Lecommandoux, S.; Tan, K. C.; de Pauw-Gillet, M.-C.; Taton, D. pH and redox responsive hydrogels and nanogels made from poly(2-ethyl-2-oxazoline). Polym. Chem. 2013, 4, 4801–4808.

(21) Bus, T.; Englert, C.; Reifarth, M.; Borchers, P.; Hartlieb, M.; Vollrath, A.; Hoeppener, S.; Traeger, A.; Schubert, U. S. 3rd generation poly(ethylene imine) for gene delivery. J. Mater. Chem. B 2017, 5, 1258–1274.

(22) Oh, S. Y.; Yoo, D. I.; Shin, Y.; Seo, G. FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. Carbohydr. Res. 2005, 340, 417–428.

(23) Barazzouk, S.; Daneault, C. Amino acid and peptide immobilization on oxidized nanocellulose: spectroscopic characterization. Nanomaterials 2012, 2, 187–205.

(24) Spackman, D. H.; Stein, W. H.; Moore, S. Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 1958, 30, 1190.

(25) Ferreira, F. V.; Pinheiro, I. F.; Gouveia, R. F.; Thim, G. P.; Bel-Hassen, R.; Boufi, S.; Salon, M. C. B.; Abdelmouleh, M.; Mariano, M.; Kissi, N. E.; Dufresne, A. Melt processing of poly(N-(2aminoethylmethacrylamide) modified Cellulose Nanocrystals: Synthesis, Characterization, and Cytotoxicity. Biomacromolecules 2015, 16, 319–325.

(26) Beck-Candanedo, S.; Roman, M.; Gray, D. G. Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. Biomacromolecules 2005, 6, 1048–1054.

(27) Oguizu, H.; Danumah, C.; Boluk, Y. Colloidal behavior of aqueous cellulose nanocrystal suspensions. Curr. Opin. Colloid Interface Sci. 2017, 29, 46–56.

(28) Reid, M. S.; Villalobos, M.; Cranston, E. D. Cellulose nanocrystal interaction probed by thin film swelling to predict dispersibility. Nanoscale 2016, 8, 12247–12257.

(29) Kloser, E.; Gray, D. G. Surface grafting of cellulose nanocrystals with poly(ethylene oxide) in aqueous media. Langmuir 2010, 26, 13450–13456.

(30) Azzam, F.; Heux, L.; Putaux, J.-L.; Jean, B. Preparation by grafting onto, characterization, and properties of thermally responsive polymer-decorated cellulose nanocrystals. Biomacromolecules 2010, 11, 3652–3659.

(31) Azzam, F.; Heux, L.; Jean, B. Adjustment of the Chiral Nematic Phase Properties of Cellulose Nanocrystals by Polymer Grafting. Langmuir 2016, 32, 4305–4312.

(32) Gray, D. G. Recent Advances in Chiral Nematic Structure and Iridescence Color of Cellulose Nanocrystal Films. Nanomaterials 2016, 6, No. 213.

(33) Davidson, P.; Penisson, C.; Constantin, D.; Gabriel, J.-C. P. Isotropic, nematic, and lamellar phases in colloidal suspensions of nanospheres. Proc. Natl. Acad. Sci. U.S.A. 2018, 115, 6662–6667.

(34) Schütz, C.; Agthe, M.; Fall, A. B.; Gordeyeva, K.; Guciuni, V.; Salajková, M.; Pliviec, T. S.; Lagerwall, J. P. F.; Salazar-Alvarez, G.; Bergström, L. Rod packing in chiral nanocellulose nanocrystal dispersions studied by small-angle x-ray scattering and laser diffraction. Langmuir 2015, 31, 6507–6513.

(35) Liu, Y.; Agthe, M.; Salajková, M.; Gordeyeva, K.; Guciuni, V.; Fall, A.; Salazar-Alvarez, G.; Schütz, C.; Bergström, L. Assembly of cellulose nanocrystals in a levitating drop probed by time-resolved small angle X-ray scattering. Nanoscale 2018, 10, 18113–18118.

(36) Uhlig, M.; Fall, A.; Wellert, S.; Lehmann, M.; Prévost, S.; Wagberg, L.; von Klitzing, R.; Nyström, G. Two-Dimensional Aggregation and Semidilute Ordering in Cellulose Nanocrystals. Langmuir 2016, 32, 442–450.

(37) Fernandes, S. N.; Almeida, P. L.; Monge, N.; Aguirre, L. E.; Reis, D.; de Oliveira, C. L. P.; Neto, A. M. F.; Pieranski, P.; Godinho, M. H. Mind the Micropag in Iridescence Cellulose Nanocrystal Films. Adv. Mater. 2017, 29, No. 1603560.

(38) Gray, D. G. Order and gelation of cellulose nanocrystal suspensions: an overview of some issues. Philos. Trans. R. Soc., A 2018, 376, No. 20170038.

(39) Bercea, M.; Navard, P. Shear dynamics of aqueous suspensions of cellulose whiskers. Macromolecules 2000, 33, 6011–6016.

(40) Nazari, B.; Kumar, V.; Bousfield, D. W.; Toivakka, M. Rheology of cellulose whiskers. Macromolecules 2000, 33, 6011–6016.

(41) Song, Y.; Zheng, Q. Linear rheology of nanofilled polymers. J. Rheol. 2015, 59, 155–191.

(42) Azzam, F.; Siqueira, E.; Fort, S.; Hassan, R.; Pignon, F.; Traelvet, C.; Putaux, J.-L.; Jean, B. Tunable Aggregation and Gelation of Thermoresponsive Suspensions of Polymer-Decorated Cellulose Nanocrystals. Biomacromolecules 2016, 17, 2112–2119.
(53) Barnes, H. A.; Hutton, J. F.; Walters, K. An Introduction to Rheology; Elsevier, 1989.

(54) Pereira, E. A.; Brandão, E. M.; Borges, S. V.; Maia, M. C. A. Influence of concentration on the steady and oscillatory shear behavior of umbu pulp. Rev. Bras. Eng. Agric. Ambiental 2008, 12, 87–90.

(55) Larson, R. G.; Magda, J. J. Coil-Stretch Transitions in Mixed Shear and Extensional Flows of Dilute Polymer Solutions. Macromolecules 1989, 22, 3004–3010.

(56) Li, M.-C.; Wu, Q.; Song, K.; Lee, S.; Qing, Y.; Wu, Y. Cellulose nanoparticles: structure — morphology — rheology relationships. ACS Sustainable Chem. Eng. 2015, 3, 821–832.

(57) Li, Y.; Li, X.; Chen, C.; Zhao, D.; Su, Z.; Ma, G.; Yu, R. Sol–gel transition characterization of thermo-sensitive hydrogels based on water mobility variation provided by low field NMR. J. Polym. Res. 2017, 24, No. 25.

(58) Abrami, M.; Chiarappa, G.; Farra, R.; Grassi, G.; Marizza, P.; Grassi, M. Use of low field NMR for the characterization of gels and biological tissues. ADMET DMPK 2018, 6, 34–46.

(59) Saito, T.; Isogai, A. TEMPO-mediated oxidation of native cellulose. The effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions. Biomacromolecules 2004, 5, 1983–1989.

(60) da Silva Perez, D.; Montanari, S.; Vignon, M. R. TEMPO-Mediated Oxidation of Cellulose III. Biomacromolecules 2003, 4, 1417–1425.

(61) Park, S.; Baker, J. O.; Himmel, M. E.; Parilla, P. A.; Johnson, D. K. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnol. Biofuels 2010, 3, No. 10.

(62) Mariano, M.; Chirat, C.; Kissi, N. E.; Dufresne, A. Impact of Cellulose Nanocrystal Aspect Ratio on Crystallization and Reinforcement of Poly(butylene adipate-co-terephthalate). J. Polym. Sci., Part B: Polym. Phys. 2016, 54, 2284–2297.