



Tracking the structural evolution of cellulose nanocrystal suspensions via small angle light scattering

Oswaldo Erazo · Nayomi Plaza · Carl Houtman · Ronald Sabo

Received: 24 September 2025 / Accepted: 18 September 2026

This is a U.S. Government work and not under copyright protection in the US; foreign copyright protection may apply 2026

Abstract Understanding and controlling agglomeration of cellulose nanocrystals (CNCs) is critical to enable their effective use in various applications. Light scattering techniques are powerful tools for studying how CNCs self-assemble in aqueous suspensions. We used small angle light scattering (SALS) and polarized microscopy to study the formation of ordered domains in CNC suspensions of moderate concentration (1–9 wt%) as a function of time and ultrasonication. Concentration, time and ultrasonication all influenced the structure and rate of tactoid formation. Ultrasonication allowed long-range order to develop faster, and at lower concentrations. The cholesteric pitch increased, and more lamellar structures were observed as sonication time increased. Our findings show that history impacts the temporal evolution of the CNC structures in solutions. These structures will likely influence the ultimate ordering of CNCs when they are used to make films or coatings.

Keywords Cellulose nanocrystal · SALS · CNC characterization · Pitch · Polarized microscopy

Introduction

Research into unique biological materials continues to attract significant interest worldwide. Developing biologically sourced materials has been challenging due to competition from man-made materials that are simpler to process and more homogenous. Despite these challenges, production of nanocellulose has emerged as a promising material. Among the various types of nanocellulose, cellulose nanocrystals (CNCs) are attractive due to their high crystallinity, excellent mechanical properties, and unique optical behavior. Primarily derived from wood pulp (Wang et al. 2023), CNCs show promise for diverse applications (Li et al. 2018; Acharya et al. 2021; Carolin et al. 2023), such as sensors (Ummartyotin and Manuspiya 2015; Singh et al. 2024), films or membranes (Douglass et al. 2018), and food packaging products (Cazón and Vázquez 2021; Liu et al. 2021; Saedi et al. 2021; Asim et al. 2022; Jiang and Ngai 2022; Li et al. 2022). CNCs are rod-shaped nanoparticles with diameters ranging from 3 to 10 nm and lengths varying between 100 and 200 nm (Kandhola et al. 2022). CNCs tend to form organized structures that can span millimeters. Understanding what factors control this structural organization is fundamental to developing products that exploit this tendency to

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10570-026-07237-3>.

O. Erazo · N. Plaza (✉) · C. Houtman · R. Sabo
USDA Forest Service, Forest Products Laboratory, One
Gifford Pinchot Drive, Madison, WI 53726, USA
e-mail: nayomi.plazarodriguez@usda.gov

O. Erazo · N. Plaza · C. Houtman
University of Wisconsin - Madison, WI, Madison, USA

become ordered and perhaps surpass the properties of traditional materials.

CNCs form highly stable colloidal structures, and naturally self-assemble into liquid crystalline phases due to the balance of attractive and repulsive forces between particles (Parker et al. 2018). CNC behavior in colloidal suspension is largely determined by particle concentration and aspect ratio. Dilute CNCs in aqueous suspensions are randomly oriented in an isotropic phase (Kádár et al. 2021). Above a critical concentration, CNCs begin to assemble into tactoids. Tactoids are anisotropic droplets that arise during isotropic–liquid crystalline phase separation in aqueous cellulose nanocrystal suspensions, appearing as ellipsoidal or spindle-shaped domains. Newly formed tactoids show a few bands, while more developed tactoids display clearer banding associated with a well-organized chiral nematic structure of the CNCs (Wang et al. 2016).

As concentration increases, a first-order phase transition from an isotropic to a cholesteric phase occurs. At even higher concentration, the suspension may become fully liquid crystalline, exhibiting a cholesteric phase, also known as a chiral nematic phase. This phase is characterized by the local alignment of CNCs and nematic order with a consistent helical twist around an axis. The distance that separates domains of nanorods with identical orientation after completing a full 360° rotation along this helical axis is known as the cholesteric pitch. Cholesteric colloidal liquid crystals can be used as templates, facilitating the development of hierarchical solid-state materials through evaporation-induced self-assembly (Parker et al. 2018). The transition between liquid crystalline order and the gel state is influenced by both concentration and interparticle attractions (Kádár et al. 2021).

To improve the dispersion quality and control the self-assembly behavior of CNCs in suspension, external mechanical treatments such as sonication are frequently applied (Metzger et al. 2021). Sonication introduces high-frequency acoustic energy into the liquid medium, causing the rapid formation, growth, and collapse of microscopic cavitation bubbles. This collapse generates intense localized shear forces and shock waves, which can break apart CNC aggregates and overcome van der Waals attractions between particles. As a result, the CNCs become more uniformly dispersed and individualized, with reduced

agglomeration (Shojaeiarani et al. 2020; Girard et al. 2021). However, it should be noted that sonication can also modify the size and shape of individual CNCs (Parton et al. 2022), and consequently, impact the self-assembly of larger structures, a behavior that is highly dependent on the aspect ratio of the CNCs.

CNC dimensions are routinely characterized with conventional microscopy techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) (Silvério et al. 2013; Chieng et al. 2017; Aguayo et al. 2018; Agarwal et al. 2021; Bushell et al. 2021), but agglomeration poses challenges (Meija et al. 2020). Combining scattering and microscopy has proven useful in improving the accuracy of microscopy-based particle sizing (Grachev et al. 2024). Small-angle neutron scattering (SANS), dynamic light scattering (DLS), static light scattering (SLS) and small-angle X-ray scattering (SAXS), have all been employed to determine the average dimensions of nanocellulose particles (Uhlir et al. 2016; Mao et al. 2017; Rodrigues et al. 2017; Azzam et al. 2020; Nagarajan et al. 2021; Grachev et al. 2024) that have been sonicated or centrifuged to some degree to improve their dispersibility and general suitability for characterization.

Although SAXS or SANS are highly effective for probing nanoscale features such as interparticle distances—which describe local packing, typically on the order of tens of nanometers (Schütz et al. 2015; Mandin et al. 2024)—it is fundamentally unable to capture larger, mesoscale periodicities that are central to the chiral organization of CNCs. In contrast, Small-Angle Light Scattering (SALS) is particularly well-suited to resolve these mesoscale features, as it directly detects structural elements on the micron scale, including the pitch of the helicoidal superstructure. The pitch is a critical parameter because it reflects the long-range chiral ordering of CNC assemblies, which governs key functional properties such as structural coloration and optical response. Likewise, the pitch reveals collective, mesoscale behavior and is highly sensitive to factors such as concentration, ionic strength or processing history due to commonly used sample preparation methods to improve dispersion such as sonication or centrifugation.

Addressing knowledge gaps with regards to behavior of CNC aggregates, rather than just individual particles, is crucial for applications where CNCs are

dispersed, since these aggregates could significantly influence material properties and performance. SALS influenced by variations in the refractive index of CNC suspensions, is particularly suited to study CNC meso-scale aggregation and large-scale phenomena such as anisotropy, particle alignment, and the structural evolution of colloidal suspensions over time (Xu et al. 2017; Kádár et al. 2021; Pignon et al. 2021). However, the technique has not been used to thoroughly study how CNC processing history influences its self-assembly behavior. Here, we combined SALS and microscopy to characterize the temporal changes in the large-scale structural organization of CNCs as a function of concentration and degree of ultrasonication and found that sonication favored the formation of well-ordered structures that became more densely packed and spanned larger areas as concentration increased.

Materials and methods

Preparation of the suspensions

CNCs were prepared at the Forest Products Laboratory as described previously (Reiner R 2013). CNC suspension concentrations of 3, 5, 7 and 9% were prepared from batch 2021-FPL-CNC-170, which has a stock concentration of 10.6 wt% CNC in water and 1.1 wt% sulfur on a dry CNC sodium form basis. A volume of 40 mL was prepared for each concentration. The CNCs were diluted in Milli-Q water and stirred thoroughly to ensure homogeneity. No additional pretreatment was applied prior to sonication. The resulting suspensions were then capped and stored in a cold room. The impact of sonication on the CNC suspensions was evaluated by subjecting the samples to sonication for 0.5 min, 1.0 min, and 1.5 min using a 700 W, 20 kHz sonication probe (Fisher Scientific FB705, Pittsburgh, USA) set at a 50% amplitude. The corresponding dispersive energies are presented in Table S1.

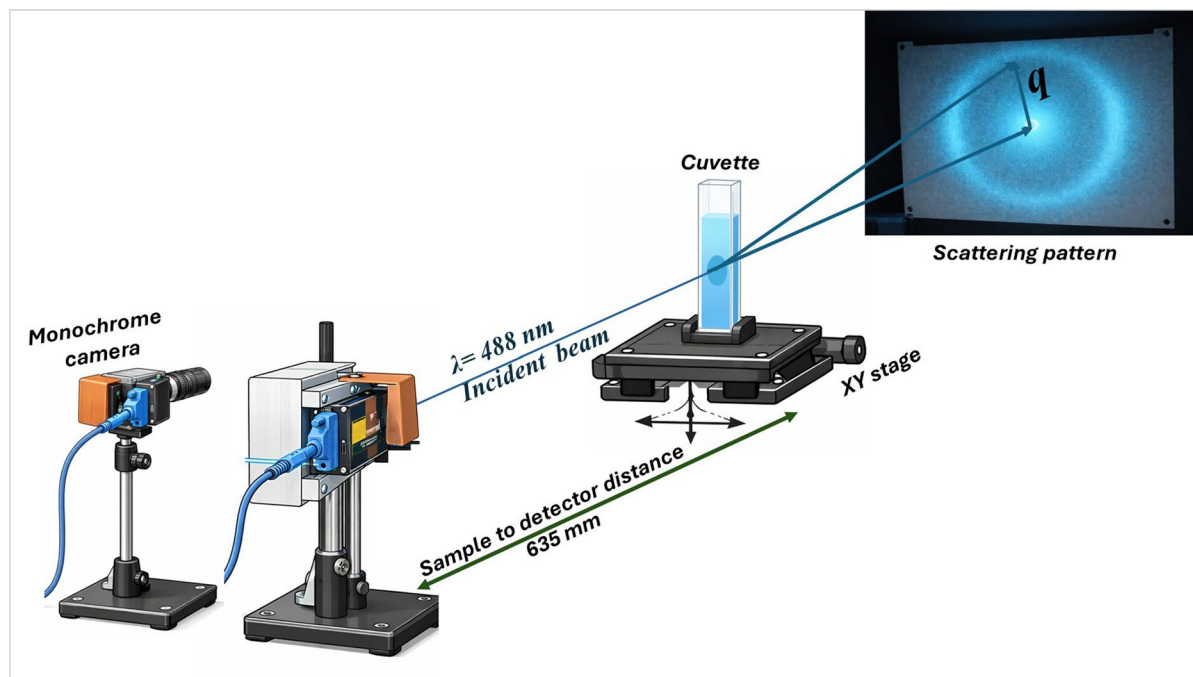


Fig. 1 Experimental setup of the SALS instrument. A cuvette containing a CNC suspension, illuminated by the light beam, generating a distinct scattering pattern

Small angle light scattering

CNC suspensions were measured using the Small Angle Light Scattering (SALS) instrument built at the USDA Forest Products Laboratory (Fig. 1). A solid-state laser with an incident wavelength (λ) of 488 nm and a power of 50mW (Spectra-Physics, Milpitas, CA, USA) illuminated a cuvette mounted on an XY stage. The spot size is approx. 0.5 mm. The sample-to-detector distance was 635 mm. An offset

the scattering vector q (Fig. 1). Measurements were taken at 0, 2, 4, 24 and 48 h. Experiments were performed at least in triplicates. All measurements were performed at room temperature and humidity conditions. Analysis focused on identifying modifications in structural organization, tracking the evolution of aggregates, and determining the influence of concentration and sonication. Data were fitted with a model comprising of a power law, a constant background and up to three Pseudo-Voigt (PV) diffraction peaks (Eq. 1).

$$I(q) = Cq^{-Pl} + \sum_{i=0}^3 \left(\frac{(1-\alpha)A_{PV}}{\sigma_g \sqrt{2\pi}} e^{\left[-\frac{(q-q_0)^2}{2\sigma_g^2}\right]} + \frac{\alpha A_{PV}}{\pi} \left[\frac{\sigma}{(q-q_0)^2 + \sigma^2} \right] \right) + B \quad (1)$$

monochrome camera (FL3-U3-13E4M, FLIR, Wilsonville, OR, USA) coupled to a C-mount adapted macro lens (12.5–75 mm, F1.8, Javelin Precision Optics, Japan) was used to acquire images of a white target using a Python 3.10 interface to the Spinnaker API (Teledyne Technologies Incorporated, Thousand Oaks, CA, USA). Three 8-bit images ranging in exposure time from 1 to 100 ms were normalized by exposure time and averaged to create a higher bit-depth composite image. A target of regularly spaced squares was imaged to determine the parameters for the biquadratic transformation needed to correct for parallax. Two-dimensional scattering patterns were collected and radially averaged to produce 1D scattering profiles with a Python 3.10 code. The accessible reciprocal space ranged from 0.15 to $8.0 \times 10^{-4} \text{ \AA}^{-1}$.

CNC suspensions were investigated in sealed quartz cuvettes (1 mm optical path length ~0.4 mL volume) to minimize multiple scattering and parasitic scattering contributions. A syringe was used to load the suspension, since it allows for precise and controlled filling of the narrow cuvette channel while minimizing the presence of air bubbles and shear inconsistencies during loading. The incident laser beam was carefully aligned and focused to pass through the mid-height of the filled cuvette, such that the same region of the suspension could be probed consistently during time-resolved measurements. Scattered light was collected at small angles to capture the intensity distribution as a function of

Here, C is a power law scaling factor, Pl is the power law exponent, and B is the background. The second and third terms of the equation represent the weighted sum of the Gaussian and Lorentzian distribution functions, respectively, with α as the parameter controlling the relative contribution of both components. Peaks are described in terms of amplitude (A), peak center (q_0), and width (σ). Pitch values (P) were determined using the formula $P = \frac{4\pi}{q_0}$, based on the first peak center.

Crossed polarizers and polarized light microscopy

The suspensions were observed by placing the cuvette between crossed polarizers to assess the overall organization and distribution of CNCs throughout. Polarized light microscopy was used to observe tactoid formation and chiral nematic phase development in CNC suspensions. To evaluate the structural homogeneity of the sample, CNC suspensions were analyzed immediately after preparation (0 h) and again at 48 h. Images of the CNC suspensions placed in cuvettes were captured using a Nikon Eclipse Ci microscope (Model 707,484, Nikon Corp., Tokyo, Japan) equipped with polarized-light optics, enhancing the visualization of intricate structural features. The imaging system included an FLIR Grasshopper 3 camera (FLIR, Wilsonville, OR, USA). Observations were conducted at a magnification of 20x, to visualize phase separation and growth.

Fig. 2 CNC suspensions at different concentrations (3, 5, 7, and 9%) observed through crossed polarizers at 0 and 48 h. Brightness increased with higher concentration due to stronger particle interactions and alignment, reflecting higher birefringence. Over 48 h, structural reorganizations were observed, with brightness decreasing or shifting, indicating dynamic rearrangements in the CNC organization over time



Results and discussion

Birefringence and polarized optical microscopy of CNC suspensions

Figure 2 illustrates the changes in birefringence of CNC suspensions observed with crossed polarizers at 0 and 48 h. Over time, phase separation becomes evident, consistent with the anisotropic–isotropic coexistence reported by Lenfant et al. (Lenfant et al. 2017). Sonication of a 3% suspension disrupts the latent aggregates formed during extended storage,

leading to a uniform isotropic texture similar to that observed at 1%, which is further supported by the isotropic scattering response in the SALS profiles (Figs. S1, S2). At 5%, non-sonicated samples showed weak birefringence at 0 h compared to the higher concentrations, which decreased further by 48 h; however, highly birefringent spots remained dispersed throughout the samples. Sonicated samples at 5% exhibited uniform brightness throughout the samples at 0 h. After 48 h, phase separation became more pronounced with increasing sonication time, and

birefringence was only detected in the chiral nematic regions.

At 7 and 9%, non-sonicated samples displayed stronger birefringence at 0 h likely due to the presence of larger CNC aggregates and stronger inter-particle interactions that enhance local anisotropy; however, sample brightness remained relatively stable after 48 h, suggesting that the initial structure was kinetically trapped and resistant to reorganization. In contrast, sonicated samples at 7% showed a gradual reduction in birefringence over time with increasing sonication time, indicating rearrangements in particle organization, which were observed in certain spots with high birefringence. At 9%, only subtle modifications in birefringence patterns were noted during

the test. This limited structural evolution may be attributed to the high particle concentration, which promotes the formation and stabilization of chiral nematic domains. The dense packing restricts further rearrangement, resulting in minimal changes over time.

The birefringence patterns provide valuable insights into the phase transitions within the suspension, and sonication seems to disrupt the structural organization of CNC suspensions. The effect of sonication on the phase evolution is further examined in Fig. 3, which presents the 5% CNC suspension observed under polarized microscopy after 48 h. The insets in Fig. 3 highlight the phase transition process observed in samples sonicated for 1.5 min. The

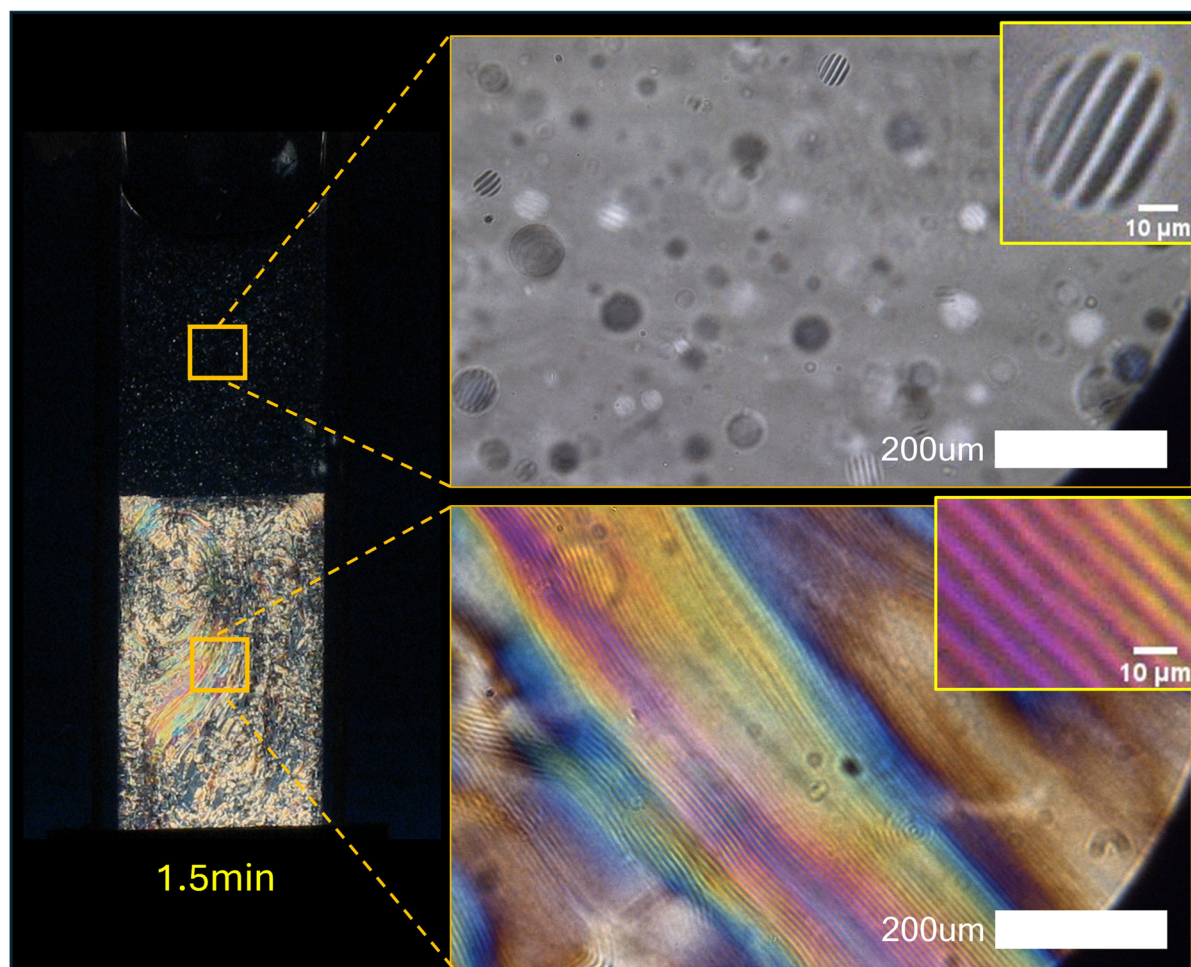


Fig. 3 CNC Suspension at 5%: The bottom inset for the suspension sonicated for 1.5 min shows a distinct anisotropic phase with a variety of interference colors and characteristic

fingerprint lines (the spacing between these lines is commonly used to measure the half-pitch), while the top inset highlights the presence of tactoids dispersed throughout the suspension

upper inset reveals the presence of tactoids, ranging in diameter from 20 to 50 μm , dispersed throughout the upper part of the suspension. Most tactoids appear as anisotropic droplets, while a few exhibit a spindle-like morphology. These tactoids appear to be in an early stage of formation, characterized by a few parallel birefringent bands. As they continue to grow or merge within the anisotropic phase, they may develop more defined band patterns, indicative of increasing chiral nematic ordering (Wang et al. 2016).

The lower inset exhibits the characteristic texture of a chiral nematic liquid crystal phase, observed here after only 48 h, thus, we observed the formation of this phase earlier than previous studies (Juárez-Rivera et al. 2021). Concentration and processing history of the CNCs is likely responsible for this difference. The size of tactoids and their likelihood of formation depends on both concentration and sonication history and consequently will determine the rate at which the tactoids evolve into the well-defined textures. Consistent with this interpretation, Fig. S3 shows that non-sonicated CNCs form these well-ordered structures at higher concentrations, whereas sonication lowers the concentration threshold required for the emergence of chiral nematic order.

While polarized optical microscopy provides direct, spatially resolved visualization of tactoid formation, phase coexistence, and local anisotropy, it is inherently sensitive to spatial heterogeneity and localized textures within the sample. As a result, POM images capture the morphology and distribution of ordered domains but do not directly quantify ensemble-averaged structural length scales or long-range ordering. In contrast, SALS measurements probe the collective scattering response from a defined illuminated volume, enabling quantitative assessment of characteristic length scales, preferred orientations, and periodic ordering even in samples that remain spatially heterogeneous. In this context, point-wise SALS measurements are particularly beneficial for capturing averaged structural organization and temporal evolution that may not be apparent from localized 2D images alone.

This distinction is directly illustrated by the corresponding 2D SALS scattering patterns. The 2D scattering pattern of the 5% CNC suspension sonicated for 1 min (Fig. 4) reveals pronounced changes in the scattering pattern after 48 h, indicating reorganization in particle alignment at larger length scales and

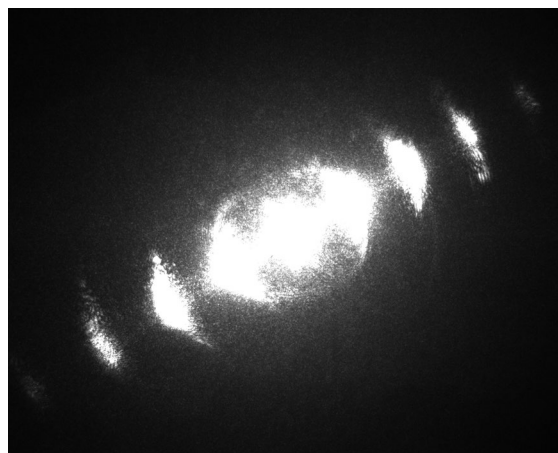


Fig. 4 2D CNC scattering pattern of a CNC suspension sonicated for 1 min. Image obtained using SALS with a solid-state laser ($\lambda=488$ nm), acquired with an exposure time of 100 ms and converted to 8-bit grayscale (Mono8)

complementing the optical observations. The emergence of localized intensity maxima reflects anisotropic scattering associated with partial CNC alignment, while the presence of anisotropic arcs indicates preferred orientations within the illuminated volume. These reciprocal-space features provide clear evidence of the development of long-range orientational order and periodic organization during temporal evolution.

Sonication effect

Figure 5 shows the scattering intensity curves for non-sonicated and sonicated CNC dispersions. The images were taken after one hour because, at low and intermediate concentrations, the shear-induced structure formed due to loading the sample using a syringe rapidly dissipates during the first hour. Beyond this period, the CNCs organization appear to evolve more slowly. At the highest concentration (9% non-sonicated), the system is highly constrained, and syringe loading does not induce a pronounced additional shear-related structure.

Figure 5a shows that at low concentration such as 3% non-sonicated, the intensity increases due to the greater number of particles present in the light path, and a peak emerges at low q ($2.17 \times 10^{-4} \text{ \AA}^{-1}$). By comparison, no peak is observed at 1% (Fig. S1), since the particles exhibit isotropic behavior, i.e.,

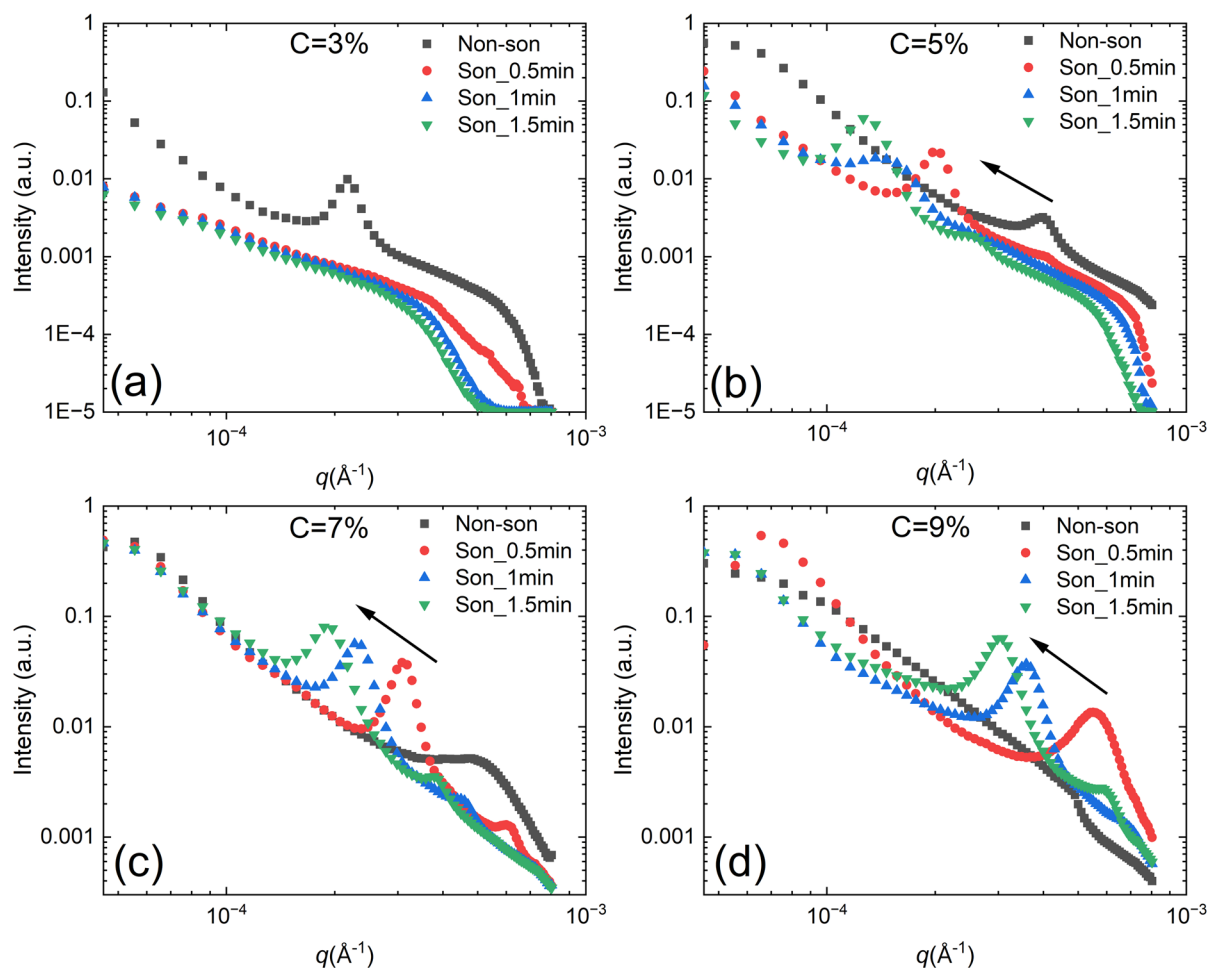


Fig. 5 Effects of sonication on the SALS from CNC suspensions measured at 1 h. **a** Non-sonicated, and sonicated for **b** 0.5 min, **c** 1 min; **d** for 1.5 min. For all concentrations, the

peak position shifts towards lower q with increasing sonication (see black arrow) indicating that the distance between particles is increasing

scattering light uniformly and exhibiting no peaks. This isotropic behavior arises from isolated nanocrystals that do not interact with each other. Sonication introduces mechanical energy that disrupts aggregate structures and modifies the spatial organization of CNCs. As a result, in the 3% sonicated samples the scattering peak disappears, and no maximum is observed in the scattering intensity profile. This behavior is consistent with the progressive breakup of clusters, which reduces the local particle number density within aggregates and increases the average interparticle spacing, leading to a more homogeneous CNC dispersion. As a consequence, the behavior of the 3% sonicated samples is similar to that of the non-sonicated samples at a concentration of 1%,

where no variation in scattering intensity is detected. Csizsar et al. observed a similar trend and reported a reduction in the size of CNC agglomerates due to sonication as a function of time (Csizsar et al. 2016) or applied energy (Shafiei-Sabet et al. 2012). In both cases, the absence of a scattering peak is associated with an insufficient particle number density to promote significant long-range interactions.

At higher CNC concentrations in non-sonicated samples (Figs. 5b–d) a peak emerges at higher q -value and progressively shifts toward higher q -value as concentration increases. The particle interactions produce structures with long-range order that lead to a structure factor peak. This shift toward higher q with increasing concentration reflects a reduction in the

characteristic distance between neighboring CNCs (Haywood et al. 2017) and indicates a more closely packed CNC arrangement, which is consistent with the formation of the cholesteric phase (Lenfant et al. 2017). In addition to reduced interparticle spacing, CNC suspensions exhibit distinct structural transitions as concentration increases. Suspensions typically transition from an isotropic phase to a biphasic structure and eventually to a gel-like state. However, increasing CNC concentration can also lead directly to a colloidal glass state without any spontaneous phase separation (Xu et al. 2017). At 5% (Fig. 5b) and 7% (Fig. 5c), partially defined peaks are observed at higher q ($4.11 \times 10^{-4} \text{ \AA}^{-1}$ and $5.21 \times 10^{-4} \text{ \AA}^{-1}$, respectively) with the broader peak at 7% approaching the lower length-scale limit of the instrument. However, at higher CNC concentrations such as 9% (Fig. 5d), the formation of larger aggregates or clusters significantly restricts particle mobility and inhibits the formation of structures with a well-defined characteristic length scale. Therefore, no peak is observed, possibly indicating a substantial reduction in pitch length that falls outside the accessible q -range.

In contrast to the behavior observed in non-sonicated samples, sonication significantly alters the scattering response at higher CNC concentrations. At 5% and 7% CNC concentrations, broader and more pronounced peaks appear, indicating the formation of ordered arrangements among the particles. The presence of these peaks in sonicated samples can be attributed to the closer proximity of particles, which facilitates ordering. The peak positions in the $I(q)$ curves correspond to characteristic distances within the sample. As CNC concentration increases, this ordering effect becomes even more pronounced. Importantly, increasing sonication time leads to a systematic shift of the scattering peaks toward lower q -value, indicating an increase in the characteristic length scale of the ordered structures. This trend is evident at both 5% and 7%, where progressively longer sonication times result in peaks appearing at lower q compared to the non-sonicated case. As CNC concentration increases, this ordering effect becomes even more pronounced. Notably, at 9%, a distinct peak emerges in sonicated samples that is absent in non-sonicated samples. Here again, increasing sonication time results in a shift of the peak toward lower q , suggesting the formation of larger characteristic structural periodicities. These observations indicate

that sonication not only promotes ordering at higher concentrations but also modifies the length scale of the resulting structures by progressively increasing interparticle spacing or domain size.

The pronounced phase separation observed in the non-sonicated 3% sample outside the initial measurement region motivated illumination of the lower part of the cuvette to evaluate the resulting structural response. Structural rearrangements in CNC suspensions evolve over time and can lead to pronounced spatial heterogeneity. In the absence of sonication, however, there are likely latent organized features related to storage of the stock solution at 10%. For this system, gravitational settling gradually drives particles toward the lower part of the cuvette, leading to distinct phase separation into biphasic and anisotropic regions after 48 h (Haywood et al. 2017; De France 2024). When observed through crossed polarizers, the upper region of the cuvette predominantly exhibits well-dispersed particles, though tactoids are also present. In contrast, the lower region displays a well-defined chiral nematic phase. Upon illumination, these phases exhibit clear differences in the SALS scattering patterns, confirming variations in structural organization (Fig. 6). However, in the 3% sonicated CNC suspension, sonication-induced structural modifications disrupt the phase separation process, preventing the formation of a well-defined anisotropic region. As a result, no distinguishable features appear in the scattering profiles.

Long-range structural evolution of CNCs over time

The temporal evolution of CNC structures measured at 1, 2, 4, 24, and 48 h, reveals distinct trends in peak formation and intensity. In the 5% CNC non-sonicated suspension, a peak emerges at $4.01 \times 10^{-4} \text{ \AA}^{-1}$, increasing in intensity and sharpening over time. In sonicated samples, the peak appears at a lower q than in non-sonicated suspensions, and this trend persists with increasing sonication time and across different concentrations. Additionally, new peaks emerge at higher q , with a second and third peak appearing after 0.5 and 1 min of sonication, respectively. However, for 1.5 min of sonication, only a second peak is observed, though it remains poorly defined (Fig. S4).

This structural evolution is further illustrated in Fig. 7a, which shows the Kratky plot of a 7% CNC non-sonicated suspension, revealing a broad,

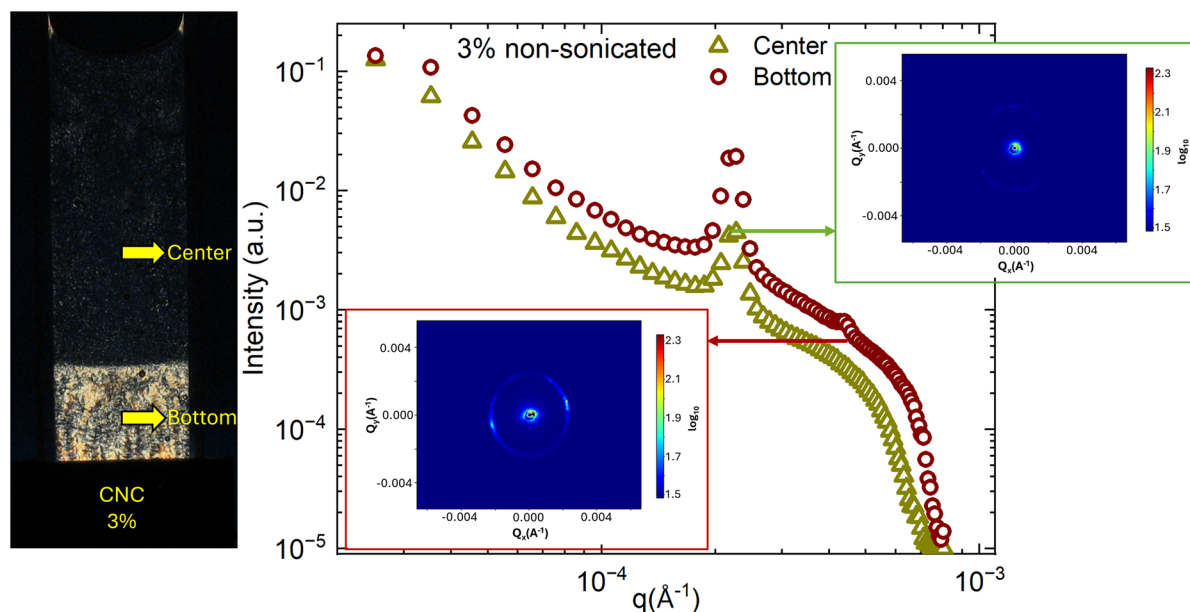
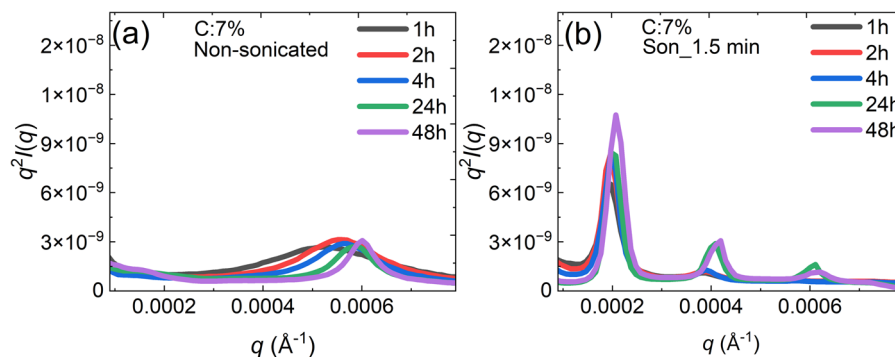


Fig. 6 1D scattering profiles of above and below the phase separation line for a 3% CNC suspension shown through cross-polarizers on the left. Corresponding 2D scattering patterns are shown as insets

Fig. 7 Kratky plot of scattering data illustrating the behavior of 7% CNC suspensions over time: **a** non-sonicated and **b** sonicated for 1.5 min. The presence of distinct peaks after sonication indicates the formation of well-defined periodic lamellar structures



low-intensity peak at $4.99 \times 10^{-4} \text{ \AA}^{-1}$, indicative of a wide distribution of CNC particle spacings. In contrast, samples sonicated for 0.5 min and 1.0 min exhibit the formation of two distinct peaks (Fig. S5). Notably, this second peak is absent in the non-sonicated suspension, suggesting long-range structural modifications induced by sonication. With prolonged sonication, these modifications become even more pronounced. Figure 7b depicts a suspension sonicated for 1.5 min where three distinct peaks appear. The positions of the three peaks indicate a highly ordered and periodic arrangement, as the spacing between them follows a harmonic

relationship: the second peak appears at $2 \mathbf{q}_1$ and the third at $3 \mathbf{q}_1$. This pattern is consistent with having a well-defined lamellar structure with long-range order. During the SALS experiment, we also observed a minor shift in peak position over time. The observed shift likely reflects gradual structural changes within the suspension, primarily driven by physical processes. Gravitational sedimentation causes CNC particles to slowly settle toward the bottom of the cuvette, leading to the development of local concentration gradients. These gradients can induce variations in the spatial arrangement and ordering of the CNCs.

At 9% CNC concentration, no discernible peak was observed throughout the test duration (Fig. S6). However, in the 0.5 min sonicated suspension, a peak appears, which is absent in non-sonicated samples. After 1.0 min of sonication, a well-defined first peak emerges alongside a broad, poorly defined secondary peak near the q range limit, resulting in an unclear peak position. For 1.5 min of sonication, two distinct peaks become evident, indicating further structural organization within the suspension. The absence of a peak indicates that there is no long-range order within the accessible q -range of the SALS. Two different scenarios could result in this absent peak: 1) a random structure, or 2) too densely packed ordered domains with a spacing that is too small to be detected.

The emergence of well-defined peaks, particularly in sonicated samples, indicates an increase in structural periodicity associated with the formation of a chiral nematic phase. The position of these peaks in reciprocal space reflects the periodic arrangement of CNCs. Specifically, the first peak corresponds to the first-order diffraction arising from the helical structure of the chiral nematic phase. The peak position was determined by fitting the scattering profiles (Fig. S7), allowing the scattering vector at the first peak to be extracted consistently. This value directly yields

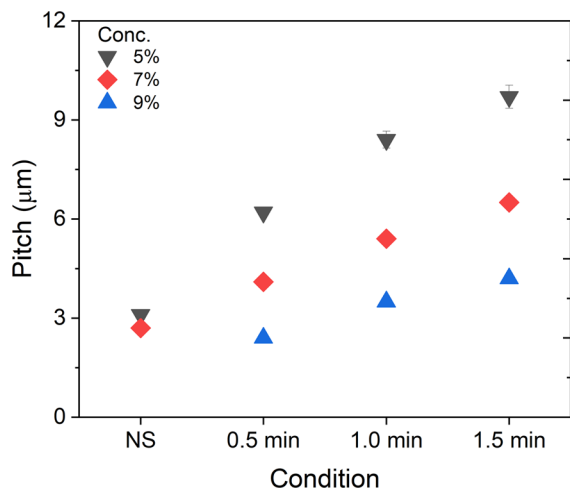


Fig. 8 Effect of Sonication time on the pitch of CNC Structures. The pitch of the chiral nematic phase decreases with increasing CNC concentration due to reduced interparticle spacing. Conversely, the pitch increases with longer sonication times, as sonication increases interparticle spacing likely by increasing the size of individual tactoids or domains with lamellar structures, and loosens the helical arrangement

the characteristic periodicity of the structure. Figure 8 shows that at lower CNC concentrations, the pitch is larger, which is attributed to wider interparticle spacing and weaker chiral interactions, a behavior consistent with findings reported in other studies (Gicquel et al. 2019; Mandin et al. 2024). As the concentration increases, the CNC particles are brought into closer proximity, enhancing chiral interactions and reducing the interparticle distance. This results in a shorter pitch, as observed in the non-sonicated samples where the pitch decreases from 3.1 μm at 5% to 2.7 μm at 7%. At higher concentrations, such as 9%, the system may become too concentrated to maintain a well-defined periodic structure, leading to the disappearance of the characteristic scattering peak and making pitch determination impossible because it would be too random. It is also possible that the system is so tightly packed that the pitch is now too small to be detected with the current instrument geometry. Future complementary studies with x-rays could provide some insight on whether there are lamellar structures spaced hundreds of nanometers apart. Likewise, adding a secondary screen in the SALS setup that is closer to the sample could expand the lower q -range.

In our experiments, the sonicated suspensions resulted in a larger pitch, reflecting a more loosely packed helical arrangement of CNCs with larger domains of lamellar structures. The increased pitch as a function of sonication time, suggests that sonication enhances particle dispersion by breaking up large aggregates and enhances the likelihood of formation of well-ordered domains (i.e. tactoids) that grow larger in size than non-sonicated CNCs. This increment in pitch size was also observed in low-concentration samples (Parton et al. 2022). The pitch values reported here are lower than those determined for volume fractions of 2.5 to 6.0 vol% (Schütz et al. 2015; Liu et al. 2019); however, our values closely align with those reported for 9% samples sonicated for 1.5 min (Pignon et al. 2021). With increased sonication time, enhanced particle dispersion disrupts large-scale alignment, resulting in a higher critical concentration for chiral nematic phase formation. Consequently, the volume fraction of the chiral nematic (CN) phase decreases, promoting the coexistence of isotropic and anisotropic phases within the suspension. Sonication also slightly elevates the CNC concentration in both the CN and biphasic regions while simultaneously increasing the pitch,

presumably by breaking large aggregates, increasing the number of particles and changing their aspect ratio. These observations are consistent with prior studies by Beck et al., who also highlighted that alterations in pitch affect the color of the final solid CNC film and explained the mechanism by which sonication may increase the chiral nematic pitch has an electrostatic origin. Protons and sulfate ions trapped within the bound-water layer (BWL) of CNCs can be released by the energy generated during sonication, limiting their return to the BWL (Beck et al. 2011; Xu et al. 2017). This disruption reduces the strength of the electrical double layer (EDL), which normally shields the chiral interaction between CNC rods. Without the electrostatic screening provided by ions in the BWL, ionic repulsion between CNCs weakens their chiral interactions, leading to an increased pitch in the chiral nematic phase (Beck et al. 2011).

Conclusions

The findings presented here deepen the understanding of CNC structural organization by showing that SALS is a powerful tool for capturing mesoscale ordering dynamics. By combining sonication, concentration, and time-resolved analysis, we identified distinct regimes of cholesteric self-assembly marked by harmonic diffraction peaks. These harmonics, not typically observed via SAXS or microscopy, offer robust evidence of well-aligned, helically ordered CNC domains. Continuous tracking over 48 h revealed the transformation of CNC suspensions from isotropic states to ordered chiral nematic phases. Given enough time, higher concentration CNC suspensions form well-organized chiral nematic structures, while lower concentration samples progress from discrete tactoids that coalesce into larger domains spanning more than 1 mm, particularly under conditions where the effective concentration increases rather than at a fixed biphasic equilibrium. We have demonstrated that SALS enables real-time tracking of this structural evolution. These findings not only advance the fundamental understanding of CNC self-organization but also offer a practical pathway for in-line quality monitoring in CNC-based manufacturing. Given the simplicity of the measurement, it is likely that an online device for monitoring CNC organization during

coating operations could be developed. Overall, this study helps bridge the gap between nanoscale characterization and macroscopic material performance.

Acknowledgements The authors thank Richard Reiner of the USDA Forest Service, Forest Products Laboratory for producing and providing cellulose nanocrystals.

Author contributions All authors contributed to the study conception and design. Methodology, formal analysis: Oswaldo Erazo, Nayomi Plaza, Carl Houtman; Writing—original draft preparation, writing—review and editing: Oswaldo Erazo, Nayomi Plaza, Carl Houtman, Ronald Sabo; validation, investigation, data curation, visualization: Oswaldo Erazo, and Nayomi Plaza; software: Nayomi Plaza, Carl Houtman; supervision, resources, funding acquisition: Nayomi Plaza, Carl Houtman, Ronald Sabo; project administration: Carl Houtman, Ronald Sabo. All authors have read and agreed to the published version of the manuscript.

Funding This work was funded by the USDA Forest Service, Forest Products Laboratory and the USDA National Institute of Food and Agriculture (Agriculture and Food Research Initiative Award No. 2023–67021-39826).

Data availability The data referenced in this study is available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Acharya S, Liyanage S, Abidi N, et al (2021) Utilization of cellulose to its full potential: a review on cellulose dissolution, regeneration, and applications. *Polymers* (Basel) <https://doi.org/10.3390/polym13244344>
- Agarwal UP, Reiner RS, Ralph SA et al (2021) Characterization of the supramolecular structures of cellulose

- nanocrystals of different origins. *Cellulose* 28:1369–1385. <https://doi.org/10.1007/s10570-020-03590-z>
- Aguayo M, Fernández A, Reyes G et al (2018) Isolation and characterization of cellulose nanocrystals from rejected fibers originated in the kraft pulping process. *Polymers (Basel)* 10:1–11. <https://doi.org/10.3390/polym10101145>
- Asim N, Badii M, Mohammad M (2022) Recent advances in cellulose-based hydrophobic food packaging. *Emergent Mater* 5:703–718. <https://doi.org/10.1007/s42247-021-00314-2>
- Azzam F, Frka-Petescic B, Semeraro EF et al (2020) Small-angle neutron scattering reveals the structural details of thermosensitive polymer-grafted cellulose nanocrystal suspensions. *Langmuir* 36:8511–8519. <https://doi.org/10.1021/acs.langmuir.0c01103>
- Beck S, Bouchard J, Berry R (2011) Controlling the reflection wavelength of iridescent solid films of nanocrystalline cellulose. *Biomacromol* 12:167–172. <https://doi.org/10.1021/bm1010905>
- Bushell M, Meija J, Chen M et al (2021) Particle size distributions for cellulose nanocrystals measured by atomic force microscopy: an interlaboratory comparison. *Cellulose* 28:1387–1403. <https://doi.org/10.1007/s10570-020-03618-4>
- Carolín F, Kamallesh T, Kumar PS et al (2023) A critical review on sustainable cellulose materials and its multifaceted applications. *Ind Crops Prod* 203:117221. <https://doi.org/10.1016/j.indcrop.2023.117221>
- Cazón P, Vázquez M (2021) Bacterial cellulose as a biodegradable food packaging material: a review. *Food Hydrocoll* <https://doi.org/10.1016/j.foodhyd.2020.106530>
- Chieng BW, Lee SH, Ibrahim NA et al (2017) Isolation and characterization of cellulose nanocrystals from oil palm mesocarp fiber. *Polymers (Basel)* 9:1–11. <https://doi.org/10.3390/polym9080355>
- Csiszar E, Kalic P, Kobol A, Ferreira EDP (2016) The effect of low frequency ultrasound on the production and properties of nanocrystalline cellulose suspensions and films. *Ultrason Sonochem* 31:473–480. <https://doi.org/10.1016/j.ultsonch.2016.01.028>
- De France KJ (2024) Chiral nematic cellulose nanocrystal composites: an organized review. *Can J Chem Eng* 102:2695–2713. <https://doi.org/10.1002/cjce.25253>
- Douglass EF, Avci H, Boy R et al (2018) A review of cellulose and cellulose blends for preparation of bio-derived and conventional membranes, nanostructured thin films, and composites. *Polym Rev* 58:102–163. <https://doi.org/10.1080/15583724.2016.1269124>
- Gicquel E, Bras J, Rey C et al (2019) Impact of sonication on the rheological and colloidal properties of highly concentrated cellulose nanocrystal suspensions. *Cellulose* 26:7619–7634. <https://doi.org/10.1007/s10570-019-02622-7>
- Girard M, Vidal D, Bertrand F, et al (2021) Evidence-based guidelines for the ultrasonic dispersion of cellulose nanocrystals. *Ultrason Sonochem*. <https://doi.org/10.1016/j.ultsonch.2020.105378>
- Grachev V, Deschaume O, Lang PR, et al (2024) Dimensions of cellulose nanocrystals from cotton and bacterial cellulose: comparison of microscopy and scattering techniques. *Nanomaterials*. <https://doi.org/10.3390/nano14050455>
- Haywood AD, Weigandt KM, Saha P et al (2017) New insights into the flow and microstructural relaxation behavior of biphasic cellulose nanocrystal dispersions from RheoSANS. *Soft Matter* 13:8451–8462. <https://doi.org/10.1039/c7sm00685c>
- Jiang Z, Ngai T (2022) recent advances in chemically modified cellulose and its derivatives for food packaging applications: a review. *Polymers (Basel)* <https://doi.org/10.3390/polym14081533>
- Juárez-Rivera OR, Mauricio-Sánchez RA, Järrendahl K, et al (2021) Shear-coated linear birefringent and chiral cellulose nanocrystal films prepared from non-sonicated suspensions with different storage time. *Nanomaterials* <https://doi.org/10.3390/nano11092239>
- Kádár R, Spirk S, Nypelö T (2021) Cellulose nanocrystal liquid crystal phases: progress and challenges in characterization using rheology coupled to optics, scattering, and spectroscopy. *ACS Nano* 15:7931–7945. <https://doi.org/10.1021/acsnano.0c09829>
- Kandhola G, Djioleu A, Rajan K et al (2022) Impact of species-based wood feedstock variability on physicochemical properties of cellulose nanocrystals. *Cellulose* 29:8213–8228. <https://doi.org/10.1007/s10570-022-04762-9>
- Lenfant G, Heuzey MC, van de Ven TGM, Carreau PJ (2017) A comparative study of ECNC and CNC suspensions: effect of salt on rheological properties. *Rheol Acta* 56:51–62. <https://doi.org/10.1007/s00397-016-0979-7>
- Li YY, Wang B, Ma MG, Wang B (2018) Review of recent development on preparation, properties, and applications of cellulose-based functional materials. *Int J Polym Sci*. <https://doi.org/10.1155/2018/8973643>
- Li J, Zhang F, Zhong Y et al (2022) Emerging food packaging applications of cellulose. *Polymers (Basel)* 14:4025
- Liu Y, Schütz C, Salazar-Alvarez G, Bergström L (2019) Assembly, gelation, and helicoidal consolidation of nanocellulose dispersions. *Langmuir* 35:3600–3606. <https://doi.org/10.1021/acs.langmuir.8b04013>
- Liu Y, Ahmed S, Sameen DE et al (2021) A review of cellulose and its derivatives in biopolymer-based for food packaging application. *Trends Food Sci Technol* 112:532–546. <https://doi.org/10.1016/j.tifs.2021.04.016>
- Mandin S, Metilli L, Karrouch M et al (2024) Multiscale study of the chiral self-assembly of cellulose nanocrystals during the frontal ultrafiltration process. *Nanoscale*. <https://doi.org/10.1039/d4nr02840f>
- Mao Y, Liu K, Zhan C et al (2017) Characterization of nanocellulose using small-angle neutron. *X Ray, Dyn Light Scat Tech*. <https://doi.org/10.1021/acs.jpcc.6b11425>
- Meija J, Bushell M, Couillard M et al (2020) Particle size distributions for cellulose nanocrystals measured by transmission electron microscopy: an interlaboratory comparison. *Anal Chem* 92:13434–13442. <https://doi.org/10.1021/acs.analchem.0c02805>
- Metzger C, Drexel R, Meier F, Briesen H (2021) Effect of ultrasonication on the size distribution and stability of cellulose nanocrystals in suspension: an asymmetrical flow field-flow fractionation study. *Cellulose* 28:10221–10238. <https://doi.org/10.1007/s10570-021-04172-3>

- Nagarajan KJ, Ramanujam NR, Sanjay MR, et al (2021) A comprehensive review on cellulose nanocrystals and cellulose nanofibers: Pretreatment, preparation, and characterization
- Parker RM, Guidetti G, Williams CA, et al (2018) The self-assembly of cellulose nanocrystals: hierarchical design of visual appearance. *Adv Mater* <https://doi.org/10.1002/adma.201704477>
- Parton TG, Parker RM, van de Kerkhof GT et al (2022) Chiral self-assembly of cellulose nanocrystals is driven by crystallite bundles. *Nat Commun*. <https://doi.org/10.1038/s41467-022-30226-6>
- Pignon F, Challamel M, De Geyer A et al (2021) Breakdown and buildup mechanisms of cellulose nanocrystal suspensions under shear and upon relaxation probed by SAXS and SALS. *Carbohydr Polym*. <https://doi.org/10.1016/j.carbpol.2021.117751>
- Reiner RRA (2013) Process scale-up of cellulose nanocrystal production to 25 kg per batch at the Forest Products Laboratory. Production and application of cellulose nanomaterials. Tappi Press, Atlanta, GA, USA, pp 21–24
- Rodrigues APH, Pereira IM, de Souza SD et al (2017) Control of properties of nanocomposites bio-based collagen and cellulose nanocrystals. *Cellulose* 24:1731–1744. <https://doi.org/10.1007/s10570-017-1218-9>
- Saedi S, Garcia CV, Kim JT, Shin GH (2021) Physical and chemical modifications of cellulose fibers for food packaging applications. *Cellulose* 28:8877–8897. <https://doi.org/10.1007/s10570-021-04086-0>
- Schütz C, Agthe M, Fall AB et al (2015) Rod packing in chiral nematic cellulose nanocrystal dispersions studied by small-angle X-ray scattering and laser diffraction. *Langmuir* 31:6507–6513. <https://doi.org/10.1021/acs.langmuir.5b00924>
- Shafiei-Sabet S, Hamad WY, Hatzikiriakos SG (2012) Rheology of nanocrystalline cellulose aqueous suspensions. *Langmuir* 28:17124–17133. <https://doi.org/10.1021/la303380v>
- Shojaeiarani J, Bajwa D, Holt G (2020) Sonication amplitude and processing time influence the cellulose nanocrystals morphology and dispersion. *Nanocomposites* 6:41–46. <https://doi.org/10.1080/20550324.2019.1710974>
- Silvério HA, Flauzino WP, Oliveira N, Pasquini D (2013) Extraction and characterization of cellulose nanocrystals from corncob for application as reinforcing agent in nanocomposites. *Ind Crop Prod* 44:427–436. <https://doi.org/10.1016/j.indcrop.2012.10.014>
- Singh S, Bhardwaj S, Tiwari P et al (2024) Recent advances in cellulose nanocrystals-based sensors: a review. *Mater Adv* 5:2622–2654. <https://doi.org/10.1039/d3ma00601h>
- Uhlir M, Fall A, Wellert S et al (2016) Two-dimensional aggregation and semidilute ordering in cellulose nanocrystals. *Langmuir* 32:442–450. <https://doi.org/10.1021/acs.langmuir.5b04008>
- Ummartyotin S, Manuspiya H (2015) A critical review on cellulose: from fundamental to an approach on sensor technology. *Renew Sustain Energy Rev* 41:402–412. <https://doi.org/10.1016/j.rser.2014.08.050>
- Wang PX, Hamad WY, MacLachlan MJ (2016) Structure and transformation of tactoids in cellulose nanocrystal suspensions. *Nat Commun* 7:1–8. <https://doi.org/10.1038/ncomms11515>
- Wang Y, Liu H, Wang Q et al (2023) Recent advances in sustainable preparation of cellulose nanocrystals via solid acid hydrolysis: a mini-review. *Int J Biol Macromol* 253:127353. <https://doi.org/10.1016/j.ijbiomac.2023.127353>
- Xu HN, Tang YY, Ouyang XK (2017) Shear-induced breakup of cellulose nanocrystal aggregates. *Langmuir* 33:235–242. <https://doi.org/10.1021/acs.langmuir.6b03807>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.