pH dependence of the chirality of nematic cellulose nanocrystals

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Cellulose nanocrystals produced by acid hydrolysis of native cellulose form a well-known chiral nematic liquid crystal phase. The mechanism involved in the formation of chirality has been the subject of a vigorous discussion. The pH and concentration dependence of the phase is studied using cellulose nanocrystal droplets within a silicon oil suspension, which allows for convenient real-time microscale manipulation of phase behaviors and properties. We demonstrate the existence of nematic phases at both low and high pH regions consistent with the Stroobants - Lekkerkerker - Odijk theory. Our results confirm electrostatic interactions play a critical role in controlling the strength of the chirality.

Cellulose, the world’s most abundant biopolymer, has attracted widespread interest throughout history for wood, paper, cloth technologies and much more modern design materials. Cellulose nanocrystals (CNCs) form liquid crystals (LCs) above a critical concentration. Generally speaking, the three factors, which determine the phase behavior of CNC LCs, are starting material, concentration and pH. Starting material allows for a good amount of control over the dimensions of CNCs. CNCs are well-known for forming the chiral nematic phase LCs that could preserve the helical orientation in solid films and serve as a template for chiral structures composed of other materials. Cellulose nanocrystals from hydrolyzing native cellulose generally present a rod-like shape with the negative charge surface and form liquid crystals (LCs) above a critical concentration. CNCs are well known for forming the chiral nematic phase LCs that could preserve the helical orientation in solid films and serve as a template for chiral structures composed of other materials.

In the present work, we prepared and characterized CNCs using established protocols, encapsulated them in silicon oil suspension and tuned the pH between 1 and 12 using 9.7 wt% sulfuric acid and 1 M sodium hydroxide. CNC LCs with tunable pH and fixed concentration showed two nematic phases in low and high pH regions. This loss of chirality in CNC LCs corresponds to a reduced Debye length and a mitigated Coulombic repulsion. The effect of electrostatic forces in CNC suspensions was analyzed and experimental results were in accordance with the theoretical analysis.

Results

Phase transition. The morphological characterization of CNCs after sulfuric acid hydrolysis (Fig. 1) is typical of these well-studied materials. Scanning electron microscope (SEM) and transmission electron microscope (TEM) images of CNCs indicate CNCs have a dimension around 5–20 nm thick and 100–200 nm long consistent with previous reports. The length distribution of the CNCs is also measured by dynamic laser scattering,
which demonstrates that the well separated individual rod-like particles are around 200 nm long (Fig. 1c). POM images of CNC LCs in bulk exhibit the nematic (Fig. 2a) and chiral nematic phase (Fig. 2b,c) with the typical Schlieren and fingerprint texture respectively. CNC LCs encapsulated in silicone oil droplets (Fig. 2d–f) allows for convenient real-time study of phase behaviors of cellulose dispersions. Silicone oil plays no noticeable role in the phase behavior itself and CNC LC phase behaviors discussed here can be observed in the bulk with no silicone oil present (Fig. 2a–c) or the droplet form (Fig. 2d–f) with same conditions, when the diameter of CNC-silicone oil droplet is above 100 μm. The initial preparation of chiral nematic CNC LC has a pH around 2 after dialysis, and the Zeta potential of CNC LCs is around −26 mV, which is below the threshold for agglomeration in CNC systems.

POM images of CNC LC droplets are shown in Fig. 3a–j and the phase diagram is presented in Fig. 3k. At a high concentration of 13 wt% (Fig. 3a–e), there exists two nematic phases at pH = 1.5 (Fig. 3a) and pH = 10 (Fig. 3e), showing the typical Schlieren texture. When pH is around 2, 3 and 5, CNCs form chiral nematic phase LCs with fingerprint textures (Fig. 3b–d). There shows a sharp transition from nematic to chiral nematic phases at both low and high pH boundaries (Fig. 3k). When the concentration of CNC LCs is lowered to 8 wt% (Fig. 3f–j), there is a slightly different sequence phase behaviors compared with 13 wt% CNC LCs. There is an isotropic phase under the POM, which means CNCs are randomly oriented without any LC order. 5 wt% CNC suspensions exhibit the isotropic phase at pH ranging from 1.5 to 11, and form the nematic again when pH below 1.5 or above 11 (Fig. 3k). Observing five batches of CNC suspensions with different concentrations (14.5 wt%, 13 wt%, 10 wt%, 8 wt%, and 5 wt%) and tunable pH (1–12), we obtain the CNC LC phase diagram describing phase transitions among isotropic, nematic and chiral nematic phase (Fig. 3k).

Pitch. POM images of chiral nematic CNC LCs (Fig. 3b–d,g,h) indicate that pH and concentration play a crucial role in pitch. 13 wt% CNC suspensions form a short pitch chiral nematic phase at pH = 2 (Fig. 3b), while at pH = 3 it is uniform chiral nematic phase with the pitch visually longer than at pH = 2 (Fig. 3c). At pH = 5 there is a mixture of long-pitch domains and domains with no noticeable chiral nematic character (Fig. 3d). 8 wt% CNC LCs form chiral nematic phases with the similar long pitch at the pH = 2 (Fig. 3g) and pH = 9 (Fig. 3h), which is reasonable since the interparticle spacing is significantly larger than the Debye length. 8 wt% chiral nematic...
CNC LCs (Fig. 3g) present a longer pitch compared with 13 wt% CNC LCs (Fig. 3b) at the same pH around 2, since lower particle concentrations allow for larger interparticle spacing and promotes the longer pitch11,19.

Discussion
The phase behavior of the rod-like suspension is determined by the particle concentration and the electrostatic properties of the solution. The four ions present in solution are $\text{H}^+$, $\text{OH}^-$, $\text{Na}^+$ and $\text{SO}_4^{2-}$ and the corresponding molar concentration $c$ can be calculated from the experimental procedure. The ionic strengths of $\text{H}^+$, $\text{OH}^-$, $\text{Na}^+$ and $\text{SO}_4^{2-}$ are calculated by $I = \frac{\sum_{i}^{n} (zc_i^2)}{2}$, where $z$ donates the charge number and calculated counter ionic strengths are shown in Fig. 4a. Total ionic strength $I$ (Fig. 4b) of CNC suspensions is calculated as $I = \frac{1}{2} \sum_{i=1}^{n} (zc_i^2)$ and rises sharply at pH below 2 and above 11 near the nematic phase boundary (Fig. 3k). Theoretical Debye length $\lambda_D = \left( \frac{2e^2}{\varepsilon \varepsilon_0} \right)^{1/2}$ is shown in Fig. 4d, where $\lambda_D$ is relatively a constant ($\lambda_D \sim 4.25 \text{ nm}$) for pH ranging from 2 to 10 while decreases rapidly when the pH goes below 2 or above 10. The electrostatic force between rod-like polyelectrolytes promotes a perpendicular orientation, and the expected phase behavior is characterized by a twist parameter $h$, which is the ratio of the Debye length $\lambda_D$ and effective diameter $D_{\text{eff}}$ of rods18,31. The effective diameter $D_{\text{eff}}$ of a charged rod is $D_{\text{eff}} = D + 5.54 \lambda_D$ in CNC suspensions, where $D$ denotes the diameter of CNC rods32. Taking $D = 12.5 \text{ nm}$ from the SEM (Fig. 1a) and TEM (Fig. 1b) images, the calculated $D_{\text{eff}}$ (Fig. 4e) shows a relatively constant $D_{\text{eff}}$ (around 37 nm) at pH ranging from 2 to 10 while decreasing sharply when pH goes below 2 or above 10. CNC LCs have a low Debye length below 2 nm (Fig. 4d), and the electrostatic twist is weakened (Fig. 4f), thus the nematic phase forms at pH below 2 and above 10 (Fig. 3a,e). At pH between 2 and 10, the large electrostatic twist (Fig. 4f) promotes the chiral nematic phase (Fig. 3b–d) over the nematic phase (Fig. 3a,e). In SLO theory, the electrostatic twist is the largest when $\lambda_{\text{D}}$ is on the order of the polyelectrolyte diameter while the electrostatic twist diminishes rapidly when the Debye length below the order of the polyelectrolyte diameter23. CNCs have sulfate half ester on their surfaces after sulfuric acid hydrolysis and the second pKa of sulfuric acid is 1.993,33, the grafted ester groups should be predominantly protonated below pH $\sim$ 2 and negatively ionic at higher pH. The phase transition of 13 wt% CNC suspensions between nematic and chiral nematic phase LCs is consistent with SLO theory. Surprisingly, there are isotropic phases between nematic and chiral nematic phase at pH around 1.5 and 10 for 8 wt%–10 wt% CNC suspensions (Fig. 3k). As the Debye length begins to decrease the chirality disappears quickly and the effective diameter $D_{\text{eff}}$ is almost three times larger than the physical D (Fig. 4e), so the Onsager criterion (The volume fraction $\varphi \sim 4D_{\text{eff}}/L$ and $L$ is the length of CNC rods) is harder to reach due to a lower effective aspect ratio. In Onsager theory, the phase separation of isotropic and LC phases is based on the entropic steric interactions and excluded volume34. Once the Debye Length is minimized the effective diameter is slightly larger than the physical diameter and the Onsager

![Figure 2](https://example.com/figure2.png)
The criterion is more easily met. The transition between nematic and chiral nematic is primarily dictated by $h$, where electrostatic interactions play a critical role in chiral helicoidal ordering in the high concentration region.

The Frank-Elastic Energy for twisting is $\nabla \times \nabla \times Kn^{1/2}$, where $K_{22}$ is the twist elastic parameter and $n$ donates the director of LCs, thus there is an entropic force that is quadratic in the displacement angle for all angles. In the chiral nematic phase region with large Debye length, considering the electrostatic interaction between a pair of charged rods we get a small angle approximation that is quadratic in the displacement angle $\beta$ (Fig. 4g). When only considering a pair-wise interaction, the Coulombic energy is minimized for orthogonal rods. Where the inter-particle separation $d$ satisfies $d = L \beta$, the interaction with the next nearest neighbor mitigates the savings associated with rotation thus enforcing an effective minimum pitch. The pitch $p$ of the chiral nematic phase is $p = 2\pi d / \beta$, combining the equation ($d = L \beta$), we get the minimum pitch should be $p = 2\pi L$ on the scale of a few microns, which is consistent with previous reports.[18,19] In chiral nematic regions with pH ranging from 2 to 10, there shows an increased ionic strength (Fig. 4c) and electrostatic forces decay faster and thus the twist interaction is decreased and the displacement angle $\beta$ between CNCs becomes smaller (Fig. 4g), leading to a larger pitch (Fig. 3b–d).

**Methods**

**Preparation of CNCs.** CNCs were prepared by the established hydrolysis protocols.[35] 6.5 g of degreasing cotton was dispersed in 70 mL of 65 wt% sulfuric acid and stirred at 46 °C in a water bath for one hour. 70 mL of deionized water was added to the resulting dispersion to quench the acid hydrolysis process. The cellulose mixture was then centrifuged in 1.5 mL tubes at 9000 rpm for 10 minutes around 5 times. This raw product was dialyzed (MWCO 12000) under static conditions for several days until the water pH reached seven, followed by 13500 rpm centrifugation for 40 minutes to purify cellulose dispersion and remove surplus deionized water to obtain CNCs that form chiral nematic LCs with pH and concentration around 2 and 14.5 wt% respectively.

**Phase behaviors and properties.** Deionized water was added into the 14.5 wt% CNC suspension to prepare four batch CNC LCs with the different concentration (13 wt%, 10 wt%, 8 wt%, and 5 wt%). Sulfuric acid (9.7 wt%) or sodium hydroxide (1 M) solution were added into 200 μL CNC LCs to make the desired pH, where the mass of adding sulfuric acid or sodium hydroxide solutions was less than 1 wt%, leading to tuning the pH without significantly changing the concentration. CNC LCs was dispersed into silicone oil and stirred for several minutes until homogeneous mixing to get relatively uniform CNC LC droplets. The pH of CNC dispersions was tracked using the pH meter (PH5S) with resolution ratio of 0.01. The concentration of CNC suspensions were
determined by weighing 200 μL of CNC suspension with an analytical balance (Mettler Toledo) followed by drying at 40 °C for 48 h to remove the solvent completely and weighing again.

Characterization and imaging. 30 μL 1.5 wt% cellulose dispersions were deposited on the ITO (indium tin oxide) glass by spin-coating at 6500 rpm for 59 s and observed under the SEM (Raith 150 TWO) at a 5 kV accelerating voltage. 0.003 wt % cellulose suspensions were dropped on copper grids, negatively stained with uranyl acetate, and observed by TEM (JEM-1200). Length distribution of the CNCs and Zeta potential of CNC LCs were

Figure 4. Calculated ionic strength of H⁺, OH⁻, Na⁺ and (a) and the ionic strength I of CNC suspensions as a function of the pH (b), where ionic strength I was increased slightly at pH = 2–10 shown in (c). Calculated Debye length λD (d), effective diameter D_{eff} (e) and twist parameter h (f) as a function of the pH. (g) Schematic of the chiral nematic phase with large displacement angle β₁ with small pitch p₁ = 2πd/β₁ (left), and chiral nematic phase with small displacement angle β₂ with long pitch p₂ = 2πd/β₂ (right). As the displacement angle β decreased (β₁ > β₂), the pitch increased (p₁ > p₂).
measured using dynamic laser scattering (Zano ZS). POM images of CNC LCs were taken using POM (Olympus BX-53M) with a rotating stage to observe the LC textures and phase behaviors.

Conclusion
We were able to tune the pH of CNC LC droplets surrounded by silicone oil at a fixed concentration from pH = 1 to pH = 12 and observed two nematic phases at low and high pH. We demonstrated electrostatic interactions played a critical role in chiral helicoidal ordering. Careful consideration of phase transitions of CNC suspensions showed that the phase behavior was consistent with the theory of charged rigid rods. Improved understanding of the physical mechanisms involved in CNC LC phase behaviors will inspire improved methods for producing templated and functional materials using CNC LC ordering.

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Author Contributions
C.L., J.E., N.W. and T.G. prepared and observed samples. J.E. and S.H. conceived the project. All authors contributed to preparing the manuscript C.L. and J.E. contributed equally.

Additional Information

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