Effect of storage conditions on cellulose nanocrystal stability

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ABSTRACT: Cellulose nanocrystals (CNCs) have evolved from a laboratory curiosity to an industrial material manufactured at a scale of up to 1 ton/day. Such large quantities of CNCs will inevitably be stored for different lengths of time before shipping and use. The chemical and physical stability of CNCs during long-term storage under various conditions was monitored. As-produced acidic H-CNCs and neutral salt form Na-CNCs were stored at ambient temperature and at 4°C as never-dried suspensions, and as a freeze-dried solid in the case of Na-CNCs. A variety of parameters were measured at intervals during the storage period. The CNC sulfate half-ester content, the cellulose chain length, and the unique optical properties of CNC films were of particular interest. Changes in these parameters were analyzed to determine the kinetics of long-term CNC degradation and establish the shelf-life of CNCs under different storage conditions.

Application: The stability and shelf-life of CNC products is of paramount importance for commercial activities involving the use of CNCs and in laboratory research, where quality, reproducible results can only be obtained if the material stability is guaranteed.

Over the last 6 years, cellulose nanocrystals (CNCs) have evolved from a laboratory curiosity produced in gram quantities for immediate use to an industrial material manufactured at a scale of up to 1 ton/day. Such large quantities of CNCs will inevitably be stored for different lengths of time before shipping and use. CNCs are an organic nanomaterial composed almost entirely of highly crystalline native cellulose. Extracted from native cellulose by sulfuric acid hydrolysis, unmodified CNCs have labile acidic sulfate half-ester groups on their surface at the locations of the primary hydroxyl groups. As such, CNCs are subject to chemical degradation, such as acid-catalyzed hydrolysis of the sulfate half-ester groups or the cellulose chains, in addition to potential bacterial and fungal growth, which may result in biodegradation of the material. It is therefore of interest and importance to determine the mechanism(s), kinetics, and effects of such degradation. The shelf-life of various CNC grades is essential information for manufacturers and consumers.

A long-term study was initiated to examine the stability of CNCs under various storage conditions. Three forms of CNCs were stored at two different temperatures (**Table I**). As-produced acidic H-CNC suspensions were titrated with sodium hydroxide (NaOH) to give the neutral salt form Na-CNCs. Samples were stored as never-dried suspensions and as a freeze-dried solid in the case of Na-CNCs. Solid H-CNCs will not be manufactured and stored for long periods and were not studied. The suspensions were stored at 23°C and 4°C; the freeze-dried Na-CNCs were stored at ambient temperature only.

Various parameters were measured at intervals during the storage period (**Table II**). The sulfur content, optical properties [1] and the cellulose chain length were of particular interest. Changes in these parameters were analyzed to determine the kinetics of long-term CNC degradation and establish the shelf-life of CNCs under different storage conditions.

Counterion	Form	Temperature, °C
	Aqueous Suspension	
H^+	4.8 wt%	23 ± 2
H^+	4.8 wt%	4 ± 1
Na^+	4.5 wt%	23 ± 2
Na^+	4.5 wt%	4 ± 1
	Freeze-dried Solid	
Na^+	95 ± 2 wt%	23 ± 2

I. Storage conditions for evaluation of long-term cellulose nanocrystal (CNC) stability.

EXPERIMENTAL METHODS

CNC extraction

Aqueous acidic H-CNC suspensions were prepared from bleached softwood kraft pulp according to a sulfuric acid hydrolysis reaction procedure modified from the literature [2]. The reaction was quenched by dilution with deionized water. After settling, the acidic supernatant was decanted and the remaining material was concentrated, washed by centrifugation, and residual acid was removed by dialysis. The concentration of the suspensions was determined gravimetrically.

Property	What Can Change It	Expected Change	Observed Change[a]
Sulfur content	Acid-catalyzed hydrolysis of sulfate half-ester groups (desulfation)	Decrease	Decrease
Suspension pH	Desulfation	Decrease	Decrease
Suspension conductivity	Desulfation	Increase	Increase
c^* for phase separation in suspension[b]	Desulfation, agglomeration, cellulose chain degradation	Increase	Increase
Particle size (by light scattering)	Desulfation, cellulose chain degradation	Increase	Very small increase
CNC film iridescence[c]	Desulfation, agglomeration	Blue-shift, iridescence loss	Blue-shift, iridescence loss, red-shift
Degree of polymerization	Acid-catalyzed hydrolysis, cellulose chain degradation	Decrease	No change

[a] In samples that underwent degradation.
[b] Critical concentration, c^*, of a CNC suspension required for self-assembly of the chiral nematic liquid crystal phase to begin.
[c] Wavelength of maximum reflection at normal incidence to the film as determined by ultraviolet-visible spectroscopy.

II. CNC properties for evaluation of long-term CNC stability.

Counterion exchange

To obtain the neutral sodium form of CNCs (Na-CNCs), dilute aqueous NaOH (0.2 N) was added to acidic CNC suspensions until a pH of 7.0 was reached.

Storage

Aqueous H-CNC and Na-CNC suspensions were stored in individual Nalgene bottles in the dark at ambient conditions (23±2°C) or in a refrigerator (4±1°C). Freeze-dried Na-CNCs were stored in individually resealable polyethylene bags in the dark at ambient conditions. The dried CNCs contained 5±2 wt% moisture adsorbed from the atmosphere.

Samples were stored for total periods of 200-373 days, depending on the characterization technique used.

Analysis of CNC samples

Sulfur (sulfate half-ester) content

Samples were diluted or re-dispersed with deionized water and extensively dialyzed using a Spectra/Por 4 membrane (Spectrum Laboratories; Rancho Dominguez, CA, USA) and 12-14 kDa molecular weight cut-off against running deionized water for at least 3 days to eliminate free sulfate ions. The samples were then lyophilized and the total sulfur content measured by elemental analysis with inductively coupled plasma–atomic emission spectroscopy (ICP-AES). The sulfur content gives an indication of the sulfate half-ester group content, assuming that all sulfur is in the form of sulfate half-ester groups covalently bound to the CNC surface.

pH and conductivity

The pH and conductivity of never-dried CNC suspensions were measured at room temperature. Measurements were taken at concentrations of 2 wt% CNCs in suspensions diluted with low conductivity (0.05 μS/cm) ultrapure (Milli-Q) water.

Phase separation and critical concentration c^* for phase separation

Suspension samples of decreasing CNC concentration were prepared by diluting the CNC suspension with deionized water. These were allowed to equilibrate in sealed glass vials at ambient conditions for several days until separation into upper isotropic and lower chiral nematic (CN) liquid crystalline phases was complete. The volume fraction of CN phase was calculated from the ratio of the height of the CN phase to the total height of suspension, and re-determined at each measurement interval during the storage period.

Particle size determination by dynamic light scattering

Particle size was determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments; Malvern, UK). Because DLS measures translational diffusion coefficients, the particle size values quoted are the z-average (intensity mean) hydrodynamic diameters of equivalent spheres and do not represent actual physical dimensions of the anisometric rodlike cellulose nanocrystals. However, they are consistent for comparison purposes. The samples were diluted or re-dispersed with deionized water to

0.1 wt% CNC and then diluted twofold with 10 mM sodium chloride. The final samples containing 0.05 wt% CNC and 5 mM sodium chloride were filtered with Whatman 0.7 μm GF/F syringe filters (GE Healthcare Life Sciences; Little Chalfont, UK) before measurement. No significant loss of CNC was measured by gravimetry after filtration. Measurements were performed in triplicate at 25°C.

Preparation of CNC films

Aliquots (15 g) of 2.5-3.0 wt% CNC suspensions were pretreated with 0-6000 J/g CNC sonication energy input using a Sonics Vibra-Cell 130 W ultrasonic processor (Sonics & Materials; Newtown, CT, USA) and 6 mm probe, at 60% amplitude, placed in polystyrene Petri dishes (84 mm internal diameter) and allowed to evaporate at 23°C and 50% relative humidity to give solid CNC films. Average film thicknesses were measured using a digital micrometer and ranged from 55-75±5 μm.

Reflection wavelength of CNC films

Films were mounted on a film holder perpendicular to the light beam in a Cary 100 Bio UV-visible spectrophotometer (Varian Canada; Mississauga, ON, Canada). The percent transmittance (%T) was measured for incidence normal to the film, with air as a reference, scanning over several locations on each film. Transmittance spectra acquisition parameters were as follows: scan range 800-200 nm, 0.1 s signal averaging time, 1.0 nm data interval, 600 nm/min scan rate, 2.0 nm spectral band width, double beam mode, and no baseline correction. The wavelength of minimum transmittance was used as an indication of the wavelength of maximum reflection.

Molecular weight distribution of cellulose chains

Samples containing 100 mg of never-dried CNCs were diluted to 0.3 wt% with ultrapure water and neutralized to pH 7 with 0.02 N NaOH at room temperature, if necessary. The CNC suspensions were then freeze-dried.

CNCs were carbanilated according to a procedure adapted from the literature [3]. Dried CNC samples (25 mg) were suspended in 10 mL of dimethyl sulfoxide (DMSO) and soaked for 1 h at room temperature, after which 1 mL of phenylisocyanate was added. After 40 h at 70°C, the carbanilation reaction was stopped by addition of 2 mL of methanol. The methanol was then evaporated for 16 h at 70°C, followed by 1.5 h at 90°C to leave the cellulose tricarbanilate (CTC) chains dissolved in DMSO.

Size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) was performed. An appropriate volume of the CTC/DMSO solution was added to tetrahydrofuran (THF) to obtain a CTC concentration of about 0.1 wt% in THF. After filtration through a 0.45 μm polytetrafluorethylene (PTFE) filter, a 50 μL of solution was injected into the SEC system. Three SEC columns (Shodex KF-805L, KF-804L, and KF-803L by Showa Denko; Minoto, Japan), preceded by a guard column, were connected in series to fractionate the CTC. The samples were eluted at 55°C at a flow rate of 0.7 mL/min. The molecular weight distribution was determined from a polystyrene calibration curve obtained using a MALS detector (DAWN DSP, Wyatt Technologies; Santa Barbara, CA, USA) coupled with a concentration detector (ultraviolet, 254 nm; Waters 486 tunable absorbance detector by Waters; Milford, MA, USA).

Property	Value
Yield, % by mass on dry pulp	46
Concentration obtained, wt% CNC	4.8
Sulfate half-ester content, mmol S/kg CNC[a]	260 ± 10
z-average hydrodynamic diameter, nm[b]	87 ± 36
*c** for phase separation, wt% CNC	3.11
Film maximum reflection wavelength, nm[c]	393 ± 10

[a] Measured by inductively coupled plasma–atomic emission spectroscopy after extensive dialysis against deionized water.
[b] Intensity mean.
[c] Films cast from 15 g of unsonicated 3 wt% H-CNC suspension.

III. Initial properties of the acidic cellulose nanocrystal (H-CNC) suspension.

Table III shows selected properties of the original never-dried H-CNC suspension used in this study.

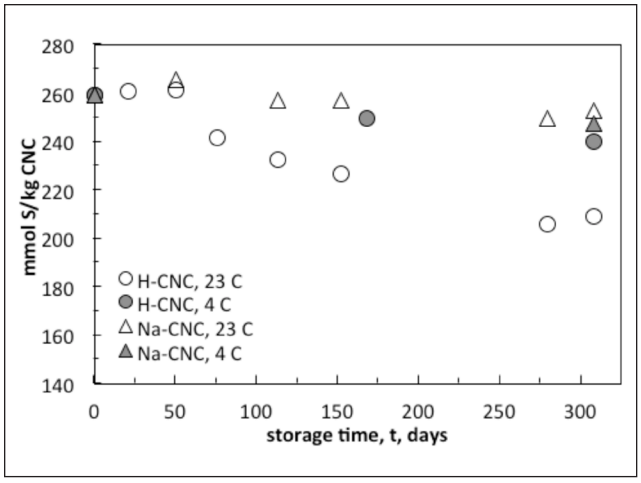
RESULTS AND DISCUSSION

Storage conditions

The pH of aqueous CNC dispersions depends on the CNC counterion. Purified CNCs produced by sulfuric acid hydrolysis have acidic sulfate half-ester groups, and are denoted H-CNCs. A 2 wt% H-CNC suspension typically has a pH of 2.3-2.9. Na-CNCs, obtained by neutralization with NaOH, are pH neutral, and therefore much more chemically and thermally stable than H-CNCs [4,5].

Cellulose nanocrystals dispersed in water or dried as a solid powder or flakes experience very different environments. For example, the proton concentration is much higher in the solid form of H-CNCs (which adsorb a small percentage of moisture, in a manner similar to paper) than in a dilute aqueous suspension of the same CNCs. One would therefore expect the kinetics of an acid-catalyzed hydrolysis reaction such as desulfation to be much faster in solid H-CNCs than in aqueous suspension. Conversely, any potential degradation or contamination caused by the growth of bacteria or fungi should be greatly slowed or eliminated in the dried CNCs.

For many reactions happening at or near room temperature, the rate of reaction roughly doubles for every 10°C rise in temperature. Storage temperature, therefore, plays a key role in determining the shelf-life of CNCs; hydrolysis reactions and fungal or bacterial growth will be slowed under refrig-



1. Sulfur content of acidic cellulose nanocrystal (H-CNCs) and neutral CNCs (Na-CNCs) stored in suspension as a function of storage time.

eration. Relative humidity, and hence sample moisture content, will also affect reaction kinetics and organism growth in the freeze-dried samples, which were assumed to have constant and equal moisture contents of 5±2 wt%.

Sulfur content

Desulfation of cellulose nanocrystals can occur by a variety of mechanisms [4,6]. In H-CNC suspensions, it occurs by acid-catalyzed hydrolysis (de-esterification). The total sulfur content of all samples was measured by ICP-AES after extensive dialysis to detect desulfation relative to the original samples. Over a period of 308 days, H-CNCs stored in suspension at room temperature lose the greatest percentage (19%-20%) of sulfur (**Fig. 1**). In contrast, neutral Na-CNC suspensions at room temperature lose only 3%-6% of their sulfur content in this period (Fig. 1). The rate of the de-esterification reaction depends on a variety of conditions, including temperature. When the suspensions are stored at 4°C, the loss of sulfur in H-CNCs is reduced to approximately that of Na-CNCs. The desulfation kinetics for H-CNC suspensions stored at room temperature are quite slow, considering that H-CNCs only appear to become colloidally unstable and precipitate from suspension at a sulfur content threshold of around 65±5 mmol S/kg CNC. The desulfation that occurs in H-CNC suspensions during storage is not sufficient to induce colloidal instability, and there is no evidence of flocculation or precipitation in the suspensions after 1 year. At a constant desulfation rate of 0.16 mmol S/kg CNC per day (**Table IV**), it would take more than 3 years at room temperature to reach the instability threshold.

Freeze-dried Na-CNCs stored at room temperature do not show an increase in desulfation rate compared to Na-CNCs in suspension (3%-5% loss of sulfur over 308 days) (Table IV). In contrast, freeze-dried H-CNCs stored for approximately 150 days lose 85% of their original sulfur content, and H-CNCs in solid film form have desulfation rates up to three orders of magnitude greater than H-CNCs in suspension (Table IV). These results confirm that Na-CNCs do not lose sulfate half-ester groups because there are no protons to catalyze desulfation by hydrolysis. Because of the rapid desulfation they undergo, storing H-CNCs in the dry state is not recommended.

Suspension pH

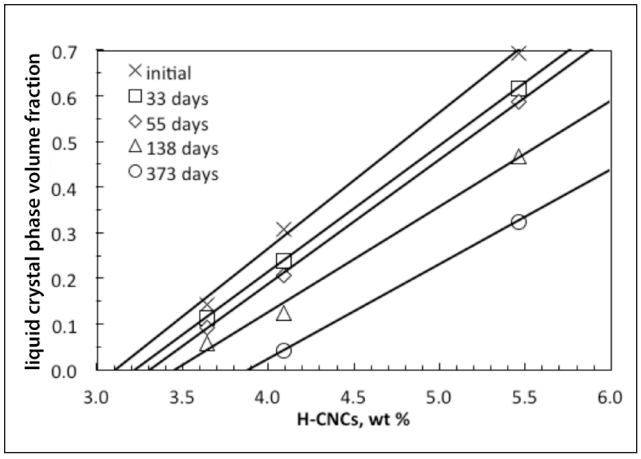
The pH (measured at 2 wt% CNCs) of the H-CNC suspension decreases slightly, from 2.76 to 2.48 over a period of 200 days at room temperature. Over time, the slow acid-catalyzed desulfation reaction generates sulfuric acid and increases the acidity of the medium. No change in pH over time is detected in H-CNC suspensions stored at 4°C. Similarly, no change in pH is observed for any of the Na-CNC suspensions because of the absence of an acid catalyst, as expected from the literature [4]. That the change in pH is the result of desulfation by acid-catalyzed hydrolysis is indicated by the correlation of the decreasing pH values with the decreasing sulfate half-ester content over time at the different temperatures.

Suspension conductivity

The conductivity (measured at 2 wt% CNCs) of the H-CNC suspension stored at room temperature increased linearly from 1251 μS/cm to 1468 μS/cm after 200 days. The increasing suspension conductivity correlates with the decreasing CNC sulfur content. No increase in suspension conductivity

Counterion	Form	T, °C	Rate, (mmol S/kg CNC)·day^{-1}	Rate Constant *k*, day^{-1}
	Aqueous Suspension			
H^+	4.8 wt%	23	0.16	7.8×10^{-4}
H^+	4.8 wt%	4	0.06	2.4×10^{-4}
Na^+	4.5 wt%	23	0.02	1.0×10^{-4}
Na^+	4.5 wt%	4	0.02	1.0×10^{-4}
	Solid			
H^+	Film[a]	23	3.27	0.2 – 0.02
Na^+	Freeze-dried	23	0.05	1.0×10^{-4}
[a] H-CNC film stored for 14 days at 0% relative humidity.				

IV. Average desulfation rates and first-order rate constants for various forms of CNCs over a period of 300 days.



2. Volume fraction of chiral nematic phase as a function of concentration for H-CNCs stored at room temperature at the storage times indicated.

was measured for H-CNC suspensions stored at 4°C, or for the Na-CNC suspensions.

Phase separation and critical concentration c for phase separation*

Highly anisometric rodlike or needlelike colloidal particles can form ordered liquid crystalline phases when their concentration reaches a certain critical value, *c** [7]. Repulsive steric and electrostatic interparticle interactions of electrically charged rods also play an important role in the ordered phase formation of rodlike polyelectrolytes such as CNCs [7,8]. For negatively charged sulfated CNCs, the electrostatic contribution to the interparticle forces depends strongly on the ionic strength of the medium [9]. Any particle agglomeration or change in the interaction forces will result in a change in the equilibrium between the liquid crystalline and isotropic phases. The CNCs' surface charge and morphology and the ionic strength of the surrounding medium will affect the phase separation of CNC suspensions into chiral nematic liquid crystal and isotropic phases [7,9,10].

Desulfation reduces the CNC surface charge and increases the surrounding ionic strength (conductivity) by releasing sulfuric acid into suspension, decreasing the repulsive force between the particles; therefore, an increase in *c** is expected for H-CNC samples [7].

Plots of the chiral nematic liquid crystal phase volume fraction as a function of H-CNC concentration show a decrease in the tendency of the ordered phase to form as storage time increases. The critical concentration *c**, given by the x-axis intercept of the plots, shifts to higher CNC concentrations (**Fig. 2**). A linear increase in *c**, from 3.11 wt% to 3.88 wt%, was observed over 373 days of storage at room temperature. The increase in *c** correlates well with the decrease in sulfate half-ester content over time, confirming that desulfation is responsible for this phenomenon.

Particle size by light scattering

We performed particle size measurements by dynamic light scattering to detect aggregation, agglomeration, or particle degradation that might occur during storage. Because the cellulose nanocrystals are rod-shaped, and the shape of the aggregates or agglomerates is unknown, the relationship between the equivalent hydrodynamic radius and the CNC particle dimensions is open to question. However, the results were comparable across the sample set. The results suggest a very slight increase of 4 nm (4.5%) in the measured z-average hydrodynamic diameter over the course of the study for H-CNCs stored at room temperature (**Table V**). Na-CNC suspensions and refrigerated H-CNC suspensions do not show detectable increases in particle size, most likely because of much slower desulfation at 4°C (Table V). No agglomeration was observed; the CNC suspensions remained colloidally stable over the timeframe of the study.

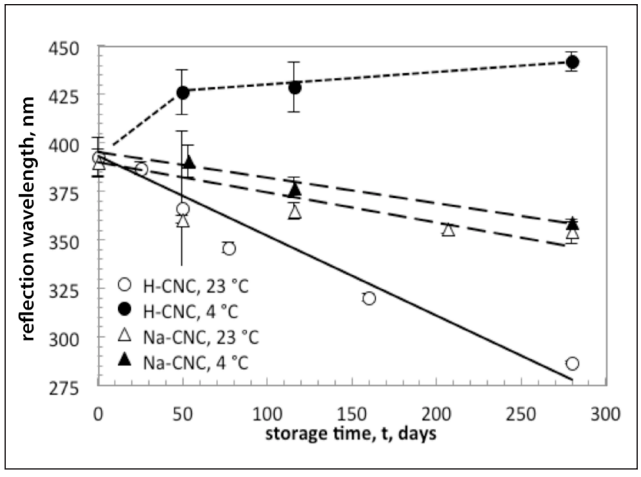
Freeze-dried Na-CNCs re-dispersed in water to 0.1 wt% showed larger particle sizes than the never-dried samples, as no sonication was applied to break up the agglomerates caused by freezing and lyophilization [11]. No increases in particle size were detected over time (Table V), indicating that no further agglomeration or aggregation occurs in the solid CNCs during storage.

Counterion	Form	T, °C	Z_{ave} Hydrodynamic diameter, nm[a]	
			t = 0 days	t > 200 days
H^+	Suspension	23	87 ± 36	91 ± 39
	Suspension	4	87 ± 36	87 ± 37
Na^+	Suspension	23	87 ± 36	88 ± 37
	Solid[b]	23	126 ± 64	123 ± 65

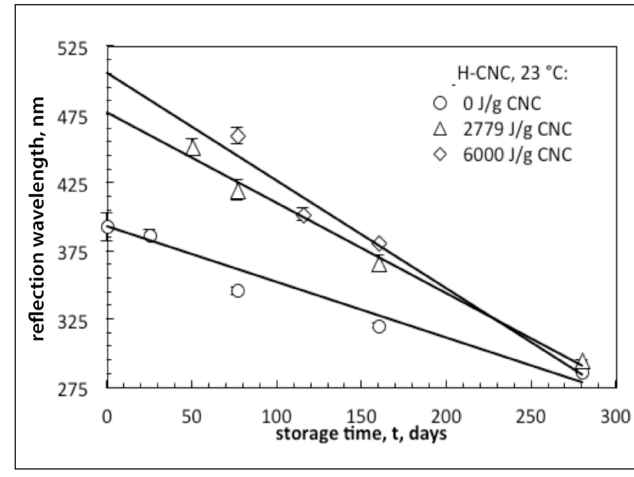
[a] The standard deviation of the Z_{ave} particle size distribution is calculated by use of a Malvern Zetasizer Nano ZS and given as the "PdI width," where PdI is the polydispersity index calculated from the cumulants analysis according to ISO 13321:1996 "Particle size analysis—Photon correlation spectroscopy."

[b] Freeze-dried Na-CNCs stored at room temperature.

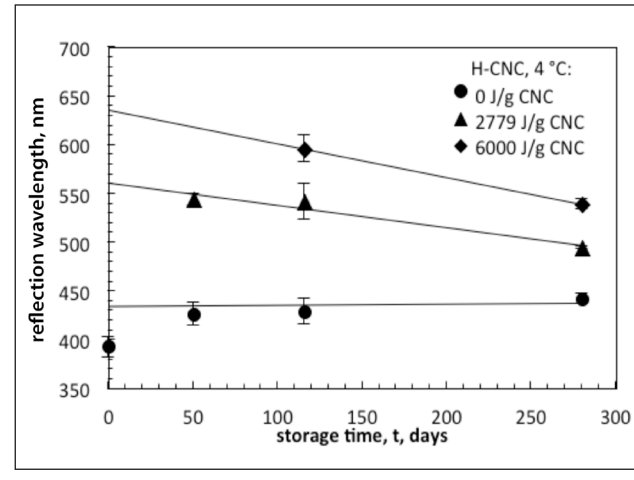
V. Initial and final z-average hydrodynamic diameters (intensity mean) measured by dynamic light scattering for CNC samples.



3. Peak reflection wavelength as a function of storage time for films cast from H-CNC and Na-CNC suspensions stored at the temperatures indicated. Error bars are standard deviations of four to five measurements across the film.



4. Peak reflection wavelength as a function of storage time for films cast from H-CNC suspensions stored at room temperature and pre-treated with the sonication energy inputs indicated. Error bars are standard deviations of four to five measurements across the film.



5. Peak reflection wavelength as a function of storage time for films cast from H-CNC suspensions stored at 4°C and then treated with the sonication energy inputs indicated. Error bars are standard deviations of four to five measurements across the film.

Reflection wavelengths of CNC films

Unsonicated suspensions of never-dried H-CNCs and Na-CNCs produce films with peak reflection wavelengths of around 390 nm (**Fig. 3**). Over time, the reflection wavelength undergoes a blue-shift toward shorter values, often detectable with the naked eye, with the exception of H-CNCs stored at 4°C, which show a small red-shift.

After 280 days, films cast from H-CNC suspensions stored at room temperature showed the most pronounced blue-shift in reflection wavelength (–106 nm), whereas Na-CNC films showed significantly smaller blue-shifts (about –35 nm, Fig. 3).

The blue-shifts in reflection wavelength over time correlate well with the CNC sulfur content and the suspension conductivity, confirming that they are caused by an increase in suspension ionic strength resulting from sulfuric acid released by acid-catalyzed desulfation [9]. Lower temperatures slow desulfation, thereby slowing down the blue-shift.

Films prepared from the H-CNC suspension stored at 4°C had reflection wavelengths that were considerably red-shifted by around +36 nm to +52 nm relative to the initial film (Fig. 3). Enhanced particle agglomeration occurring at the lower temperature might increase the pitch, outweighing the simultaneous blue-shift caused by desulfation. Blue-shifts in reflection wavelength induced by very small sonication energy inputs (30-80 J/g CNCs) before film casting support this hypothesis, as this treatment de-agglomerates the CNC particles; larger energy inputs (230-300 J/g CNCs) lead to the expected sonication-induced red-shifts [1]. The red-shift in films prepared from refrigerated H-CNC suspensions appears to gradually increase during extended storage at 4°C (Fig. 3).

Effect of sonication on CNC film reflection wavelength

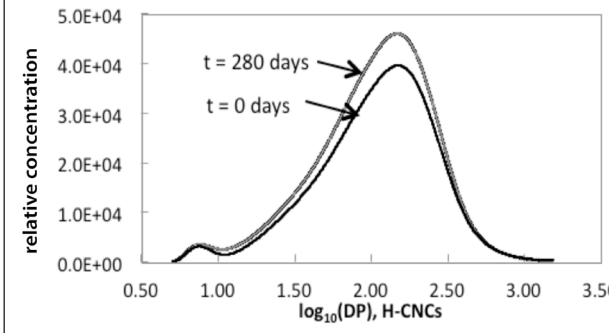
Ultrasound treatment of CNC suspensions increases the chiral nematic pitch in suspension, causing a red-shift in the reflection wavelength of CNC films with increasing applied energy. The red-shift results from a mechanism that involves reducing the concentration of ions trapped near to the CNC surface, allowing the electrical double layer to better screen the chiral interactions between nanocrystals or increasing the excluded volume of the nanocrystals and their associated oligosaccharide gel layer [1]. The sensitivity of the film reflection color development with sonication can be described in terms of nanometers of red-shift per joules of energy applied per gram of CNC (nm·[J/g CNC]$^{-1}$).

Figure 4 shows the reflection wavelength of films cast from H-CNC suspensions stored at room temperature and treated with increasing sonication energy inputs. All curves show a decrease in film reflection wavelength (blue-shift) with suspension storage time. However, the sensitivity to

Counterion	T, °C	Sensitivity[a], nm·(kJ/g CNC)$^{-1}$		
		t = 0 days	t = 50 days	t = 280 days
H^+ [b]	23	19	16	~0
Na^+ [c]	4	18	18	17

[a] For 6000 J/g CNC sonication energy input.
[b] Calculated from regression lines in Fig. 4.
[c] Calculated from unreported data.

VI. Sensitivity of red-shift to sonication in films cast from CNC suspensions stored for various lengths of time and then sonicated.



6. Molecular weight (DP) distributions for H-CNC suspension stored at room temperature.

sonication decreases with storage time and was eliminated after 280 days (Fig. 4). When H-CNCs are stored at 4°C their sensitivity to sonication is much less affected by storage time (**Fig. 5**). **Table VI** summarizes the sonication sensitivity of CNC suspensions after storage at various conditions.

While a slight overall blue-shift with storage time is observed for unsonicated (Fig. 3) and sonicated CNC suspensions (Fig. 4), Na-CNCs stored at room temperature lose much less sensitivity to sonication over time, and Na-CNCs stored at 4°C do not appear to lose sensitivity at all (Table VI). Films cast from suspensions of re-dispersed freeze-dried Na-CNCs are not iridescent when the suspensions are not pretreated with sonication energy; the red-shift in reflection wavelength induced by increasing sonication of the re-dispersed freeze-dried Na-CNC suspensions does not diminish over time. Over time, a reduction in sensitivity to sonication is caused by the increasing suspension ionic strength because of sulfuric acid released by H-CNC desulfation, which counteracts the electrostatic changes caused by the application of ultrasonic energy [1]. The associated loss of CNC surface charge (sulfate half-ester content) will also contribute to the blue-shift in the films [12,13]. This is supported by the retention of sonication sensitivity in neutral Na-CNCs, which do not undergo desulfation (Table VI).

Molecular weight distribution of cellulose chains

If the cellulose chains in CNCs undergo depolymerization by acid-catalyzed hydrolysis, the molecular weight distribution measured by SEC-MALS should shift to smaller values. No change in molecular weight distribution over time was ob-

Counterion and Form	T, °C	Target Property	Shelf-life	Expected Change
H-CNC (aq)	23[a]	Film reflection wavelength	2 months	≤35 nm blue-shift
		Film color development by sonication	2 months	≤20% sensitivity lost
		Colloidal stability of suspension	3 months	≤10% S content lost
H-CNC (aq)	4[b]	Film reflection wavelength	10 months	≤25 nm red-shift
		Film color development by sonication	4 months	≤20% sensitivity lost
		Colloidal stability of suspension	10 months	≤10% S content lost
Na-CNC (aq) and (s)	23[a], 4[b]	Film reflection wavelength	1 year +	≤35 nm blue-shift
		Film color development by sonication	1 year +	~0% sensitivity lost
		Colloidal stability of suspension	1 year +	≤5% S content lost

[a] Not recommended because of the possibility of microbial growth in suspension.
[b] Do not freeze; freezing causes particle agglomeration [11].

VII. Recommended storage conditions and shelf-life for different forms of CNCs.

served for any of the samples. The molecular weight distributions showed a weight-average degree of polymerization (DP_w) of 140-150 initially and after 280 days (**Fig. 6**). The nature of the small shoulder peak (low molecular weight component) seen in molecular weight distributions of CNCs will be addressed in a future report.

Based on the results of this study, the acidic protons in the H-CNC suspensions that catalyze the hydrolysis of the sulfate half-ester groups are not sufficient to cleave cellulose chains at room temperature and below. Despite the observed growth of fungi or bacteria in the room temperature samples and some of the refrigerated samples after 5-6 months, we can conclude that bacterial or fungal degradation does not occur in the CNC cellulose chains. The high crystallinity of the cellulose chains most likely renders them inaccessible to microbial attack. No microbial growth was observed in the freeze-dried Na-CNC samples over the course of the study.

Storage conditions, shelf-life and stability monitoring

Table VII lists recommended storage temperature and suggested shelf-lives for different forms of CNCs, based on the results of this study. The optimal shelf-life for a given form of CNCs will depend on the exact properties required for the target application. The appropriate shelf-life may vary for a given storage condition because properties degrade at different rates, particularly for H-CNCs.

Containers should be tightly sealed when not in use and CNC suspensions should be handled with appropriate precautions to avoid fungal or bacterial contamination. We recommend that all forms of CNCs be stored under refrigeration (4±1°C), because degradation reactions and bacterial and fungal growth are limited at lower temperatures. However, CNC suspensions should not be frozen, as this induces particle agglomeration [11].

The simplest method for monitoring the aging of CNC suspensions is to measure their increase in conductivity from desulfation over time. This method can be quite sensitive, depending on the CNC concentration. However, the decrease in chiral nematic phase volume fraction of an H-CNC suspension with a concentration greater than c^* is even greater in terms of the percentage change in the measured variable over time.

SUMMARY AND CONCLUSIONS

This work has demonstrated that within a 1-year storage period, degradation in CNC properties can occur, the extent of which depends on the storage conditions, CNC counterion (acidic vs. neutral), and the CNC state (aqueous suspension vs. freeze-dried solid). Significant degradation occurs in aqueous acidic H-CNC suspensions stored at room temperature. Minor changes are observed in H-CNC suspensions stored under refrigeration, in neutral Na-CNC suspensions stored at room temperature and under refrigeration, and in freeze-dried solid Na-CNC.

ABOUT THE AUTHORS

The effect of storage conditions is of great importance to many members of the cellulosic nanomaterial community, from researchers in the laboratory who require measurements made on samples that are stable over time, to producers and companies using these materials, who require stable products with reproducible and predictable properties.

Although cellulose nanocrystals (CNC) desulfation at harsher conditions and shorter periods (hours to days) had been examined, the evolution of CNC chemical stability at typical storage conditions over extended periods (weeks to months), and its effects on the suspension self-assembly properties had not been investigated. These properties are what make CNCs unique and attractive for use in a wide range of products and applications.

The biggest challenge of this research was ensuring that the CNC suspensions did not become contaminated with microorganisms over the year-long testing period, which would have interfered with interpretation of the results. To avoid this, immediately after production, each suspension was sealed in individual sample containers, which were only opened at the time of testing.

The most interesting finding was that although fairly concentrated suspensions of acidic CNCs underwent autocatalyzed desulfation quite slowly at room temperature, the effect of this on the self-assembly properties was noticeable relatively early on in the study. CNCs are very sensitive to their environment.

Pre-commercial and commercial producers of CNCs will benefit directly from our findings because they can use this information to properly store the material they produce to provide their customers with high-quality, reproducible CNCs.

The next step is to examine the specific effects of CNC aging on particular applications and find ways to avoid or minimize any problems it might cause.

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Most of the expected changes in CNC behavior associated with this degradation were observed during our study (Table II). During storage, the phase separation behavior and self-assembly properties in aqueous suspension, including CNC film reflection wavelength and its development with sonication energy input, underwent the most change, particularly in H-CNC suspensions stored at room temperature.

The degradation of the above properties is linearly correlated with the CNC sulfate half-ester content. During prolonged storage, H-CNCs undergo acid-catalyzed desulfation, losing 5% to 20% of their original surface sulfate half-ester group content. The rate of the desulfation reaction is controlled by the CNC proton content; desulfation is much slower or is eliminated in Na-CNC samples. Refrigeration also slows the desulfation significantly, thereby slowing the degradation of many other properties. Decreasing pH and increasing conductivity are also indicative of desulfation.

The mean value of the CNC particle size measured by dynamic light scattering did not change significantly over the period of this study, indicating that minimal agglomeration occurs during storage. Cellulose chains in the CNCs do not depolymerize under the storage conditions studied.

Neutral Na-CNCs are stable and can be stored for up to 1 year with minimal degradation. Because H-CNCs undergo desulfation, they should be stored only in suspension at 4°C. Their shelf-life depends on the specific properties needed for the end use: H-CNCs intended for optical applications requiring controlled development of self-assembled chiral nematic structures may be used after 3 months or less, whereas they may still be used after 10 months to 1 year if only a stable colloidal suspension of nanometric sized particles is required (e.g., for further compatibilization). **TJ**

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