

Auto-catalyzed acidic desulfation of cellulose nanocrystals

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SUMMARY: The surface charge density of cellulose nanocrystals (CNCs) extracted by sulfuric acid hydrolysis is determined by its anionic sulfate half-ester content. Sulfate half-ester moieties are relatively labile, and can be removed by acid- or alkaline-catalyzed de-esterification at high catalyst concentration and temperatures above 50°C. Auto-catalyzed desulfation in acidic (H-CNC) suspensions also occurs slowly at ambient conditions. Desulfation poses a problem because loss of surface charge alters CNC suspension stability, liquid crystal properties and rheological behaviour. The rate of acid-catalyzed desulfation depends on the local concentration of sulfate half-ester groups with proton counterions at the surface of the cellulose nanocrystal. High temperatures and low moisture content significantly increase the reaction rate constant. Temperatures just above 0°C substantially slow desulfation in H-CNC suspensions during long-term storage. Neutral salt forms of CNCs do not undergo significant *in situ* desulfation even under very harsh conditions.

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At the commercial scale, cellulose nanocrystals (CNCs) are typically extracted from bleached chemical wood pulps by sulfuric acid hydrolysis. During the hydrolysis reaction, anionic sulfate half-ester groups are grafted at the sites of hydroxyl groups on the cellulose nanocrystal surface. Cellulose nanocrystals from wood pulp usually contain between 240 and 330 mmol sulfur per kg CNC (Beck-Candanedo et al. 2005; Araki 2013). Repulsion between their negatively charged surfaces controls CNCs' colloidal stability and dispersion in water and other polar solvents, as well as their chiral nematic liquid crystal self-assembly and rheological behaviour (Araki 2013).

Desulfation (the loss of sulfate half-ester groups and their replacement by hydroxyl groups) of CNCs leads to a reduction in surface charge and destabilizes aqueous CNC suspensions. Below a threshold surface charge density, attractive van der Waals forces dominate and cellulose nanocrystals agglomerate or precipitate (Jiang et al. 2010; Araki 2013). The acidic sulfate half-ester content also affects the thermal stability of CNCs (Roman, Winter 2004; Wang et al. 2007), which is improved by neutralization with hydroxide bases.

Several different methods have been used to desulfate cellulose nanocrystals produced by sulfuric acid hydrolysis; these methods are summarized in Table 1.

Heating aqueous suspensions of cellulose nanocrystals which have protons as counterions (H-CNC) causes an *in*

situ acid-catalyzed hydrolysis (desulfation) reaction. Dong and Gray (1997) heated a 7.6 wt% H-CNC suspension to 60°C and monitored its phase separation into isotropic and anisotropic (chiral nematic) liquid crystalline phases; the volume fraction of the anisotropic phase decreased from 0.60 to 0.15 over 6 days and then remained stable at this value. The suspension pH was also found to decrease due to the generation of sulfuric acid. The sulfuric acid increased the ionic strength of the surrounding medium, screening the remaining CNC surface charges and decreasing the volume fraction of the liquid crystal phase. It should be noted that the CNC surface sulfur contents were not determined in these experiments. Desulfation in H-CNC suspensions becomes significant at temperatures of around 40–50°C (Dong, Gray 1997).

Mild hydrochloric acid hydrolysis has been used to desulfate polysaccharides (Jiang et al. 2010). While it is difficult to achieve complete desulfation in one mild HCl treatment, the extent of desulfation can be controlled by successive repetition of the treatments. Jiang et al. (2010) heated cellulose nanocrystals (1 wt%) in 25 mM HCl at 80°C for 2.5 h and then removed the excess acid. The process was repeated seven times. The nanocrystal sulfur content decreased by about one-third of the original amount during the first four treatments, and then stabilized in a manner similar to the volume fraction of anisotropic phase when heating H-CNC suspensions (Dong, Gray 1997). The reversibility and possible stereoselectivity of the de-esterification reaction are likely responsible (Jiang et al. 2010).

Solvolytic desulfation involves heating the pyridinium salt of a polysaccharide in dimethyl sulfoxide (DMSO) in the presence of methanol or water (Jiang et al. 2010). In contrast to mild HCl hydrolysis, one treatment step removes nearly all the sulfur groups (Jiang et al. 2010).

Sodium hydroxide-catalyzed de-esterification (saponification) has also been used to desulfate CNC suspensions and films with heating. Heating a 9 wt% CNC suspension in 2 M NaOH at 65°C for 5 h was found to remove all sulfate half-ester groups (Hasani et al. 2008), while milder conditions (2.87 wt% CNCs in 1 M NaOH at 60°C for 5 h) resulted in aggregation of the particles and a decrease in sulfate half-ester content from 240 to 80 mmol/kg (Kloser, Gray 2010). Using still milder conditions, Habibi et al. (2010) claim that “sulfate groups ... [can] be eliminated by alkaline treatment” of Langmuir-Schaeffer films of cellulose nanocrystals deposited on gold surfaces (0.01–1 M NaOH at flow rates of 0.2 ml/min for 20 min at 20°C), although the sulfate half-ester contents were not determined.

Spherical cellulose nanocrystals prepared by mixed HCl and H₂SO₄ acid hydrolysis have a low initial sulfate half-ester content (< 20 mmol/kg CNC) as determined by conductometric titration (Wang et al. 2007). Wang et al. (2007) claim to have removed these sulfate half-ester groups by (i) washing the CNCs with deionized (DI)

Table 1 - CNC desulfation methods reported in the literature.

Method	Conditions	mmol S/kg CNC ^a		Reference
		Initial	Final	
Acidic desulfation	H-CNC suspension, > 40–50°C, days	94.7	-- ^b	Dong, Gray 1997
	25 mM HCl, 80°C, 2.5 h	293	191	Jiang et al. 2010
	x 3		103	
	x 4		58	
	x 7		55	
Solvolytic desulfation	DI water wash, 23°C, 1 month	16.7	< 2.0	Wang et al. 2007
	CNC pyridinium salt in DMSO with methanol, 80°C, 2 h	293	7	Jiang et al. 2010
Alkaline desulfation	2 M NaOH, 65°C, 5 h	130±95	-- ^b	Hasani et al. 2008
	1 M NaOH, 60°C, 5 h	240	80	Kloser, Gray 2010
	0.01–1 M NaOH, 0.2 ml/min, 20°C, 20 min	237–265	-- ^b	Habibi et al. 2010
	pH 9, 23°C, 48 h	16.7	0	Wang et al. 2007
	~1.0% NaOH, 23°C, 48 h	16.7	0	Wang et al. 2007

^a Sulfur contents measured by conductometric titration; ^b No sulfur contents measured or given in the publication.

water for more than a month; (ii) keeping the suspension at pH 9 for 48 h; or (iii) diluting the suspension 20-fold with 1.0% NaOH and letting it stand for 48 h at room temperature

There is an important caveat to consider when determining sulfate half-ester content: most of the sulfate half-ester contents discussed above were found indirectly by conductometric titration of the acidic sulfate half-ester groups against a dilute base. Total sulfur determination by elemental analysis or inductively coupled plasma–atomic emission spectroscopy (ICP-AES) tends to give higher sulfate half-ester contents (Dong et al. 1998; Araki et al. 1999). When performing conductometric titration, one must ensure that all sulfate half-ester groups are fully protonated by pre-treating the CNCs with an acid-form ion exchange resin (e.g. Kloser, Gray 2010). Even when the CNCs have been purified with deionized water, full protonation may not occur. While ICP-AES analysis eliminates the need for protonation, it assumes all sulfur is in the form of sulfate half-ester groups located at the cellulose nanocrystal surface.

Strong mechanical energy treatments such as sonication do not result in detectable desulfation (Beck et al. 2011), indicating that there is a strong covalent bond between the CNC surface and the sulfate half-ester. As a result, desulfation kinetics are slow at room temperature even in concentrated acidic H-CNC suspensions. It appears that high concentrations of acid or base in conjunction with some heat are necessary to accelerate the desulfation reaction to significant levels (Table 1). Heating H-CNC suspensions without additional acid is preferable to hydrolysis using added strong acid, as glycosidic linkages in sulfated polysaccharides are often susceptible to acid hydrolysis, increasing the risk of depolymerization (Jiang et al. 2010).

Industrially produced CNCs may be stored for long periods under a range of conditions, during which time any acidic proton counterions present may catalyze a “self-desulfation” reaction. This report examines the desulfation of both aqueous suspensions and dried forms of acidic and neutral CNCs, including the dependence of CNC desulfation kinetics on temperature, concentration, and pH. *Note:* The term “sulfur content” refers to sulfur covalently bound to the cellulose nanocrystals.

Materials and Methods

Preparation of CNC suspensions

An aqueous H-CNC suspension (5.5 wt%) was prepared in the FPInnovations CNC pilot plant using air-dried commercial fully bleached softwood kraft pulp (Kappa number < 0.5) according to a procedure modified from the literature (e.g. Dong et al. 1998). Our typical hydrolysis conditions are: 60–65 wt% sulfuric acid at 4–65°C for 25–45 min of reaction. The final suspensions were purified by centrifugation (Sorvall Legend XTR Centrifuge) at 10,000 rpm for 15 min at 10°C. The total CNC sulfur content was measured as mmol S/kg CNC by ICP-AES. To obtain neutral salt-form Na-CNCs, acidic CNC (H-CNC) suspensions were titrated with dilute aqueous NaOH (0.2 N, Sigma-Aldrich) to a pH of 7.0.

Preparation of CNC films

Aliquots (15 ml) of 2.75 wt% CNC suspensions were placed in polystyrene Petri dishes (84 mm internal diameter) and evaporated at 23°C and 50% relative humidity (RH) until solid CNC films (70 ± 10 µm thick, Fowler IP54 digital micrometer) were obtained.

Preparation of freeze-dried CNCs

CNC suspensions (~5 wt%) were frozen at –80°C and then lyophilized (VirTis Freezemobile 12SL) under < 50 mTorr vacuum at room temperature to give a flaky solid.

Measuring CNC sulfur/sulfate half-ester contents

After dialysis or washing, CNC samples were freeze-dried and analyzed for total sulfur content by ICP-AES. Alternatively, purified CNC suspensions were fully protonated by treatment with Dowex Marathon C strong acid cation-exchange resin (H⁺ form) and the acidic sulfate half-ester groups determined by conductometric titration (Metrohm Titrando) against 0.02 M NaOH.

Desulfation of aqueous CNC suspensions by acidic de-esterification

CNC suspensions were stored in sealed containers at room temperature or at 4°C for up to 1 year, or heated in sealed glass containers at 70–85°C for up to 33 days. A 2.7 wt% H-CNC suspension was also treated with 0.1 N

HCl (Sigma-Aldrich) at room temperature for 24 h with stirring. Excess sulfuric and hydrochloric acid was removed by dialyzing the suspensions (Spectra/Por 4 membrane, 12–14 kDa MWCO) against running DI water for at least 3 days.

Desulfation of aqueous CNC suspensions by alkaline treatment

CNC suspensions (0.55–2 wt%) were heated at 23 to 50°C in 0.10 or 0.85 N NaOH solutions for 20 min to 3 h. The mixtures were then neutralized to pH 5–6 with 3 N HCl. After dialysis, the sulfur content was determined by ICP-AES.

Desulfation in solid H-CNC films

H-CNC films (freshly cast from a 3.2 wt% suspension) were stored for up to 30 days at 23°C and relative humidities of 0% (vacuum desiccator), 50% (controlled environment) or 100% (airtight box containing water). The films stored at 50 and 100% RH were allowed to redisperse for several h in 20 ml of purified milliQ water (18.2 MΩ·cm). The films stored at 0% RH were not redispersible; they were cut into small pieces and soaked for several h in 20 ml of milliQ water. After soaking, all samples were mixed by vortex at 3,000 rpm for 30 s. The resulting suspensions and redispersed films were dialyzed against running DI water for a minimum of 3 days, freeze-dried and total S determined by ICP-AES.

Freshly cast H-CNC films were heated to 35, 40, 50, 80 and 105°C for different lengths of time. The non-redispersible films were then cut into small pieces and rinsed with 50 ml of DI water (pH ~5, conductivity 0.6 μS/cm) up to 10 times each. Typically, the pH of the rinse water increased from 3 to 5 and the conductivity decreased from 700 to 1 μS/cm, respectively, after 6 or 7 rinses. The films were then freeze-dried and the total S content determined by ICP-AES.

Desulfation in freeze-dried H-CNCs

After both the freezing and the sublimation steps of lyophilization, CNC sulfur contents were measured by ICP-AES after dialysis. Freeze-dried H-CNCs were also stored in a sealed plastic bag at ambient conditions for 5 months and the sulfur content determined. Repeated freeze-drying and dialysis was also carried out on one sample without measuring the sulfur content between cycles.

Results and Discussion

Desulfation of aqueous CNC suspensions by in situ acidic de-esterification

Although auto-catalyzed desulfation kinetics in pure CNC suspensions at ambient conditions are slow, they are not negligible. Aqueous suspensions of H-CNCs (5.5 wt%) and Na-CNCs (5.0 wt%) suspensions were stored at room temperature (~23°C) and 4°C and the sulfur content monitored. The results are summarized in Fig 1. After storage at room temperature for 308 days, the H-CNC suspension contains 193 mmol S/kg CNC, a 19.8% decrease from the initial sulfur content of 240 mmol/kg, whereas the H-CNC suspension stored at 4°C contains 221 mmol S/kg CNC, a decrease of only 8.1%.

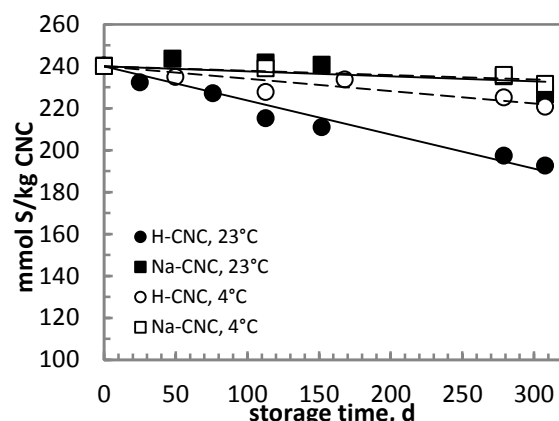


Fig 1 - Sulfur contents for H-CNC (5.5 wt%) and Na-CNC (5.0 wt%) suspensions stored at 23 and 4°C.

Lower temperatures clearly reduce the acid-catalyzed desulfation reaction rate, slowing the desulfation of the acid-form CNCs to nearly that of the neutral salt form.

Over the same time period, the Na-CNC suspensions stored at both temperatures show minimal desulfation, losing only 5.7% and 3.5% of their initial sulfur content at 23 and 4°C respectively, confirming the acid-catalyzed nature of the desulfation mechanism.

The above results are somewhat in contradiction with those of Wang et al. (2007), who report a 90% loss of sulfur after a month of repeated washing with distilled water (Table 1). The apparent almost complete desulfation observed by Wang et al. (2007) during prolonged washing cannot be explained by self-catalyzed acidic de-esterification; we detected a maximum of 5% sulfur loss by elemental analysis in CNC suspensions which were dialyzed for 30 days against running DI water. Considering the very low initial sulfate half-ester content (16.7 mmol S/kg CNC) quoted by Wang et al. (2007), it is likely that their conductometric titration measurements were performed on sodium-form CNCs that had not first been fully reprotonated, resulting in erroneous determinations of the sulfate half-ester content.

Heating H-CNC suspensions to temperatures of 70°C and above greatly increases the desulfation reaction rate. Around 65% of the sulfur is removed after 3 to 5 days of heating at 70 and 85°C, and after only 2 h at 100°C (Table 2), leading to significantly lower surface charge densities. Conductivity measurements confirm this; the unheated and 85°C H-CNC samples have conductivities of 450 and 60 μS/cm, respectively, after dialysis. As expected, Na-CNC samples undergo minimal or no desulfation under these conditions.

Table 2 - Sulfur contents for CNC suspensions heated at high temperatures.

Sample	wt% CNC	T, °C	time	mmol S/kg CNC	
				Initial	Final
H-CNC	3.8	70	5 d	265 ^a	90 ^a
Na-CNC	3.8	70	5 d	265 ^a	254 ^a
H-CNC	2.8	85	3 d	275 ^b	103 ^b
Na-CNC	2.8	85	3 d	275 ^b	275 ^b
H-CNC	4.0	100	2 h	217 ^a	108 ^a

^a Measured by ICP-AES.

^b Measured by conductometric titration.

The time scale of desulfation changes dramatically as the temperature is increased: Whereas it takes a 5 wt% H-CNC suspension 10 months at 4°C to lose 8% of its sulfur content; it takes around 14 weeks at 23°C, 7 h at 70°C and only 30 min at 90°C. When refrigerated, H-CNC suspensions are nearly as stable to desulfation as neutral Na-CNC suspensions, which is an important consideration for industrial applications which may require the use of never-dried H-CNCs.

Effect of CNC suspension concentration on rate of *in situ* acidic de-esterification

A mechanism for the surface esterification (sulfation) of cellulose nanocrystals during 64 wt% sulfuric acid hydrolysis has been given by Lu and Hsieh (2010), although the esterification reaction product is missing an oxygen atom between the C6 carbon and the sulfur atom. *In situ* desulfation of pure H-CNCs proceeds by reversible acid-catalyzed ester hydrolysis. It is important to note that in this case there is no free sulfuric acid present in our systems, which consist only of sulfated cellulose nanocrystals suspended in water. The de-esterification reactions must occur at the CNC surface within the bound water layer (BWL). Desulfation is therefore self-catalyzed by the proton counterions associated with the sulfate half-esters grafted to the CNC surface. This implies that:

- Diffusion of the proton counterions and the sulfuric acid released by desulfation is limited to the region close to the CNC surface (BWL);
- Local proton concentrations at individual CNC surfaces are more important than the bulk proton concentrations throughout the suspension.

At higher temperatures, desulfation kinetics are much more rapid, allowing the reaction to be studied in more detail. Fig 2 shows the decrease in sulfur content over time for H-CNC suspensions of increasing concentration at 70°C. The initial fast desulfation rate during the first 2 days or so appears to be similar for all CNC suspension concentrations. Although desulfation is rapid initially, it levels off or slows significantly after around 5 to 10 days for all suspensions, analogously to the decrease in anisotropic phase volume fraction in heated H-CNC suspensions of increasing concentration observed by Dong et al. (1997). The loss of sulfur after 33 days of heating varies from 55% for the 1 wt% suspension to 80% for the 9 wt% suspension.

In the absence of free acid, proximity of surface sulfate half-ester groups to each other is necessary to catalyze the de-esterification. This can be considered an “intra-CNC” reaction, as the proton of an acidic sulfate half-ester may catalyze the hydrolysis of an adjacent sulfate half-ester group at the CNC surface. As the CNCs undergo desulfation, the released sulfuric acid diffuses into the bulk water surrounding the nanocrystals, where it is too dilute to shift the reaction equilibrium toward sulfation (Hamad, Hu 2010). The degree of sulfation (DS) decreases, leading to lower sulfate half-ester density at the CNC surface. Once a threshold CNC sulfur content is reached, the surface proton counterion density at the CNC surface ($\text{R-OSO}_3\text{H}$) falls below a critical value required for effective self-catalysis of further desulfation.

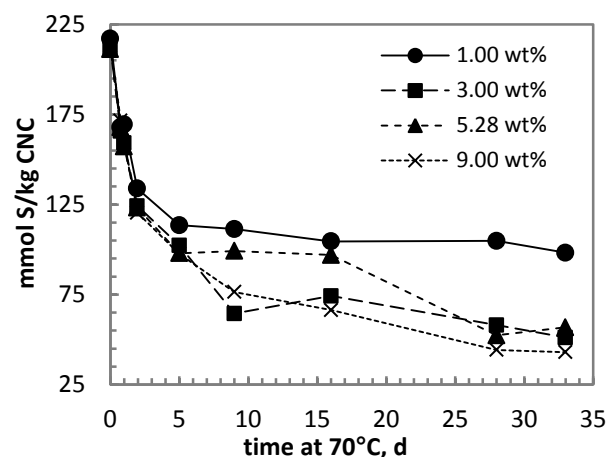


Fig 2 - Sulfur contents for H-CNC suspensions heated at 70°C.

Table 3 - Colloidal stability of suspensions of CNCs with decreasing sulfur contents.

h at 90°C	mmol S /kg CNC	Before dialysis ^a		After dialysis ^b	
		Clarity	Gel	Clarity	Ppt
0	209	+	-	+	-
10	104	+	-	+	-
24	70	-	+	+	-
36	58	-	+	-	+
48	48	-	++	-	++
72	37	-	+++	-	+++

^a Desulfated CNCs (~ 1 wt%) containing released H_2SO_4 .

^b Pure desulfated CNCs (< 1 wt%).

That is, the remaining sulfate half-esters are no longer in close enough proximity for intra-CNC desulfation and a plateau is eventually reached (Fig 2). We thus propose that CNCs effectively contain two types of sulfate half-esters – those that are removed more easily and rapidly in the initial stages of desulfation, and those that remain as the reaction continues and somewhat levels off, indicating very slow kinetics.

As the CNC suspension concentration increases, the level-off DS decreases from 100 mmol S/kg CNC at 1 wt% CNC to 40 mmol S/kg CNC at 9 wt% CNC (Fig 2). At higher concentrations, two CNCs may approach each other closely enough for an acidic sulfate half-ester on one to catalyze the hydrolysis of a sulfate half-ester on the other; this “inter-CNC” catalyzed desulfation is more likely as CNC concentrations increase. As the CNC sulfate half-ester content decreases with desulfation, “inter-CNC” catalysis occurs less frequently, leading to a plateau in a manner similar to that described above. However, the combination of “inter-CNC” and “intra-CNC” desulfation reactions decreases the DS at which the “level-off” point of desulfation is reached at longer heating times.

Effect of desulfation on colloidal stability of CNCs in suspension

The sulfur content of CNCs extracted by sulfuric acid hydrolysis is often used to predict the colloidal stability of nanocrystal suspensions, as the surface charge determines the interparticle repulsion forces (Araki et al. 1998; Jiang et al. 2010; Kloser, Gray 2010). The colloidal stability of CNC suspensions desulfated by heating to

90°C was observed both before and after dialysis to determine the lowest sulfur content at which CNCs are electrostatically stabilized in water. Table 3 summarizes the results. Suspensions of CNCs which have been heated for 24 h and contain 70 mmol S/kg CNC appear cloudy and contain macroscopic gel particles upon dilution with DI water prior to dialysis. After dialyzing to remove the H₂SO₄ released by desulfation, a stable colloidal suspension is recovered, indicating that the colloidal instability of the pre-dialysis suspension was due to the higher ionic strength medium created by the excess H₂SO₄ (Dong et al. 1996; Revol et al. 1997; Araki et al. 2001; Boluk et al. 2011; Zhong et al. 2012). In contrast, suspensions of CNCs which have 58 mmol S/kg CNC behave similarly prior to dialysis, but remain cloudy after dialysis and yield some white precipitate after centrifugation. Finally, a CNC suspension having 37 mmol S/kg CNC is cloudy after dialysis, and separates into a clear supernatant above a white precipitate upon centrifugation.

There appears to be a threshold sulfur content of around 65 ± 5 mmol S/kg CNC below which precipitation begins in pure CNC suspensions; full precipitation appears to occur at around 45 ± 5 mmol S/kg CNC.

Desulfation of aqueous CNC suspensions by alkaline de-esterification

When alkaline de-esterification conditions (0.10–0.85 M NaOH, pH 13.0–13.9 at room temperature or 50°C) are applied to 0.5–2 wt% CNC suspensions and followed by extensive dialysis, no significant desulfation is observed (Table 4). Within experimental error, all the sulfur contents are identical before and after treatment. These experiments confirm the stability of Na-CNC suspensions at 50°C.

The NaOH solutions and/or CNC suspensions listed in Table 4 may be too dilute for significant de-esterification to occur; the only successful alkaline desulfations in the literature for which sulfur content data is given are performed with 1–2 M NaOH and CNC concentrations of greater than 3 wt% (Hasani et al. 2008; Kloser, Gray 2010). Additionally, the reaction times investigated in Table 4 may be too brief and/or the temperatures too low. Given these results, it is doubtful whether the conditions used by Habibi et al. (2010) in Table 1 actually desulfate the CNCs, unless the flow (0.2 ml/min) of reagent solution strongly shifts the reaction equilibrium in the direction of desulfation.

Table 4 - Sulfur contents for CNC suspensions treated with alkaline conditions.

M NaOH	wt% CNC	T, °C	t	mmol S/kg CNC ^a	
				Initial	Final
0.1	2	23	20 min	234	222
0.1	0.56	50	20 min	244	240
			160 min	244	225
0.85	0.55	50	20 min	240	228
			180 min	240	229

^a Measured by ICP-AES after dialysis.

In situ desulfation of solid acidic H-CNC films

The rate of self-catalyzed H-CNC desulfation is higher in the solid state (e.g. CNC films and freeze-dried or spray-dried material) than in aqueous suspension. Solid CNC films are an extreme case of increasing the CNC suspension concentration: Passing from aqueous suspension to the solid state accelerates desulfation by significantly increasing local acidic sulfate ester proton counterion concentrations at CNC surfaces due to their very close proximity to each other.

Solid films of cellulose nanocrystals adsorb water from the atmosphere in a manner analogous to pulp fibers in paper. The moisture content of the films is distributed throughout the film structure, surrounding the CNCs, and including a bound water layer in which the water molecules are bound directly to the surface of the cellulose nanocrystals. Bound water on cellulose has been detected by calorimetry (Stamm, Loughborough 1934), density measurements (Filby, Maass 1932) and by ¹H-NMR spectroscopy of moist cellulose (Carles, Scallan 1973). It does not evaporate at moderate pressure and temperature conditions even in an environment having 0% relative humidity; CNC films will still contain some moisture under these conditions, allowing hydrolysis to proceed.

As Fig 3 shows, the relative humidity (RH) of the environment in which H-CNC films are stored strongly affects the desulfation rate and extent. After 32 days, the H-CNC film stored at 0% RH loses half of its initial sulfur content whereas the film stored at 100% RH loses only 12%. In contrast to the solid films, the reference suspension (3.2 wt% H-CNC) containing ~ 97% water reaches a plateau rapidly and loses only 3% of its initial sulfur content in the same time period. No plateau is apparent for the CNC films at these conditions over this time period.

There are a constant number of proton counterions associated with attached sulfate half-ester groups for a given sample of pure CNCs containing no residual dissolved ionic material. In suspension, protons and sulfate anions released by desulfation are free to diffuse

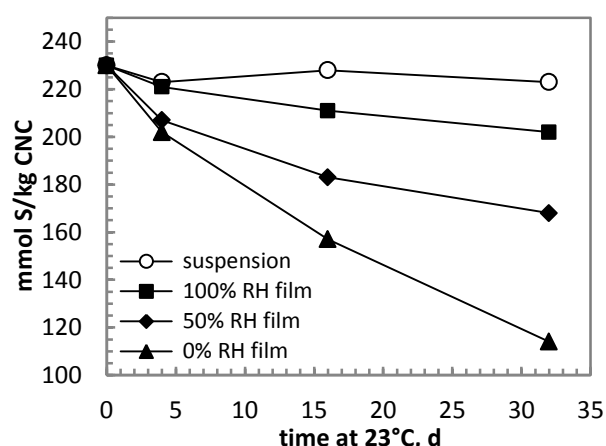


Fig 3 - Sulfur contents for solid H-CNC films at room temperature and decreasing relative humidity. Reference = H-CNC suspension (3.2 wt%).

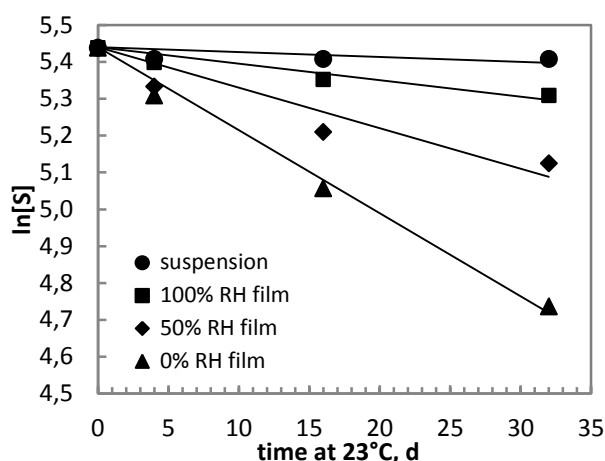


Fig 4 - Plots of $\ln[S]$ as a function of time for solid H-CNC films at room temperature and decreasing relative humidity. Rate constants k : $1.3 \times 10^{-3} \text{ d}^{-1}$ (suspension), $4.5 \times 10^{-3} \text{ d}^{-1}$ (100% RH), $1.1 \times 10^{-2} \text{ d}^{-1}$ (50% RH), $2.25 \times 10^{-2} \text{ d}^{-1}$ (0% RH).

into the bulk water surrounding the nanocrystals. In contrast, in solid CNC systems, protons (sulfate half-ester counterions and released sulfuric acid) are distributed throughout the much smaller volume of adsorbed water surrounding the CNCs, resulting in a significantly higher proton activity at the CNC surface. Additionally, “inter-CNC” catalyzed desulfation is much more important in solid CNCs due to the greatly increased proximity of the nanocrystal BWLs and the sulfate half-ester groups.

As the relative humidity of the surrounding environment decreases, the water layer at the CNC surfaces will evaporate and become thinner, further increasing local proton activity and increasing the proximity of neighbouring CNCs and their sulfate half-esters. At 0% RH, only the bound water layer present at the CNC surface provides a medium for the desulfation reaction; the proton concentration and CNC proximity, and hence the desulfation rate, are highest at these conditions.

A plot of the logarithm of H-CNC sulfur content as a function of time (Fig 4) shows that the desulfation reaction is first-order in total CNC sulfur content, and consequently first-order in the proton concentration in the bound water layer. As expected, the desulfation rate constant at 0% RH is 5 times greater than at 100% RH, and 17 times greater than in suspension.

In contrast to H-CNC films, neutral salt form Na-CNC films stored for 2 years at ambient conditions lose only 3–4% of their sulfur content (Table 5).

Effect of temperature on in situ desulfation of solid acidic H-CNC films

Heating solid H-CNC films further accelerates desulfation due to the combined effects of increased temperature, and the elimination of some adsorbed water, which increases the acid concentration at the nanocrystal surface (the bound water layer does not evaporate at the temperatures studied). Table 5 summarizes these results.

The sulfur loss increases more than 3-fold from 6% for a freshly-cast film to 21% for an identical film after 18 h in a desiccator. When the temperature increases to 50°C, 55% desulfation is observed after 18 h. At 80°C, more

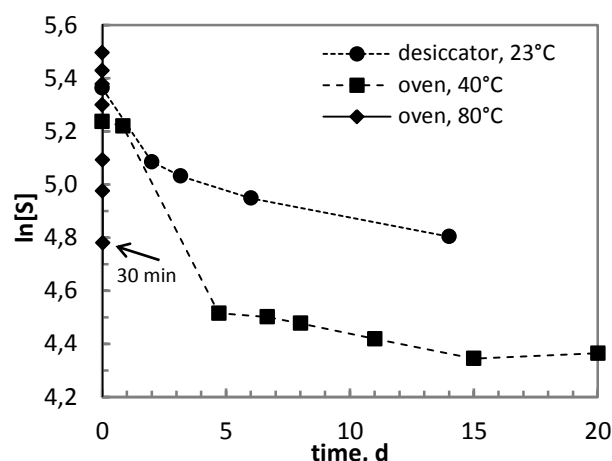


Fig 5 - Plot of $\ln[S]$ as a function of time for solid H-CNC films in ~0% relative humidity environments at 23, 40 and 80°C.

Table 5 - Sulfur contents of solid CNC films after heating.

Form	T, °C	Location	t	mmol S/kg CNC ^a		
				Initial	Final	% S lost
Na	23	Ambient	2 yrs	244	233	4
H	23	Ambient	< 1 h	244	230	6
H	23	Desiccator	18 h	244	193	21
H	35	Oven	24 h	244	167	32
H	50	Oven	18 h	244	109	55
H	80	Oven	30 min	244	119	51
H	105	Oven	5 min	244	81	67
Na	105	Oven	6 h	229	231	--

than half the sulfur is removed after 30 min, whereas at 105°C fully two-thirds of the sulfur content is eliminated after only 5 min (Table 5). We did not follow desulfation at 80 and 105°C in H-CNC films for longer times, due to the onset of other reactions such as cellulose dehydration and typical sulfuric acid blackening of carbohydrates.

The level-off DS appears to decrease with increasing temperature (Fig 5). Higher temperatures increase the desulfation reaction rate and decrease the adsorbed water content, which increases surface proton activity. This may counterbalance the simultaneously increasing free sulfate concentration, resulting in more significant desulfation at level-off.

Our results tend to contradict the statement made by Dong et al. (1997) that H-CNCs dried in a vacuum oven at 35°C for 24 h undergo negligible desulfation; almost a third of the sulfur was removed under similar conditions in this study. Heating a dilute ($\leq 10 \text{ wt}\%$) H-CNC suspension in a sealed container at 35°C for short periods most likely does not result in significant desulfation (Fig 1); however, once the water evaporates, the desulfation rate increases dramatically even at ambient conditions (Fig 4).

In the absence of an acid catalyst, solid CNCs are very stable to desulfation: heating Na-CNC films at 105°C for up to 16 h does not cause significant desulfation (Table 5).

When H-CNC films are kept in 0% RH environments at 23°C or heated to 40°C, plots of $\ln[S]$ as a function of time show an initial sharp drop before the reaction rate

begins to decrease after around 2 to 5 days (Fig 5). The initial desulfation rate constants are similar at both temperatures ($k = 0.17\text{--}0.22\text{ d}^{-1}$). However, the initial rate constant k is over two orders of magnitude larger at 80°C (53.7 d^{-1}).

Our results suggest that due to the very small volumes of water present in solid CNC systems, the sulfuric acid released during desulfation has a significant effect on the reaction equilibrium as it progresses. Initially, the high local proton concentration due to CNC proximity outweighs the effect of the simultaneously increased free sulfuric acid concentration. However, as the DS decreases, the sulfuric acid accumulates near the CNC surfaces due to its limited diffusion range.

The slowing and leveling off of the desulfation reaction in heated CNC films is likely due to sulfuric acid becoming concentrated enough to shift the equilibrium toward the reactants ($\text{R-OSO}_3\text{H}$ and water), slowing the desulfation.

Revol et al. (1997) suggest that progressive desulfation caused by heating solid H-CNC films reduces their dispersibility by allowing greater interparticle bonding. Later work (Beck et al. 2012) shows that the moisture content of freshly cast H-CNC films controls their dispersibility in water, but does not provide evidence regarding the contribution of desulfation to loss of dispersibility. Desulfation and the presence of protons may partially contribute to the loss of dispersibility of H-CNCs by allowing greater interparticle bonding at relatively higher moisture contents than in Na-CNCs. Although it is impossible to isolate the effects of moisture removal (drying) and desulfation for dried H-CNCs, neutral salt-form CNCs provide a clue: Na-CNC films become non-dispersible in water when heated at 105°C for longer than 16 h, but do not undergo detectable desulfation, strongly suggesting that moisture content, and not desulfation, controls the dispersibility of dried CNCs.

Desulfation in freeze-dried CNCs

Slow drying of H-CNC suspensions by evaporation at ambient conditions results in significant desulfation after 1 or 2 days. Freeze-drying is another method used to obtain solid CNCs, in which the suspension is first frozen at very low temperatures (-80°C) and then sublimated under vacuum while still frozen ($T < 0^\circ\text{C}$). Table 6 summarizes the effect the steps of lyophilization have on the sulfur content of H-CNCs. Freezing alone appears to cause a measurable amount of desulfation, despite the low temperatures and short processing time. The sublimation process has fast desulfation kinetics and causes an additional 40% loss of sulfur despite the low temperatures which are expected to slow the reaction kinetics. Freeze-drying causes a similar amount of desulfation to film casting ($\sim 10\text{--}15\%$ S lost immediately). The desulfation kinetics in freeze-dried H-CNCs over long storage times are also similar to the desulfation kinetics of solid H-CNC films (see Fig 3 and Fig 5). The extent of desulfation after 5 months (85%) suggests that the system may be approaching complete desulfation.

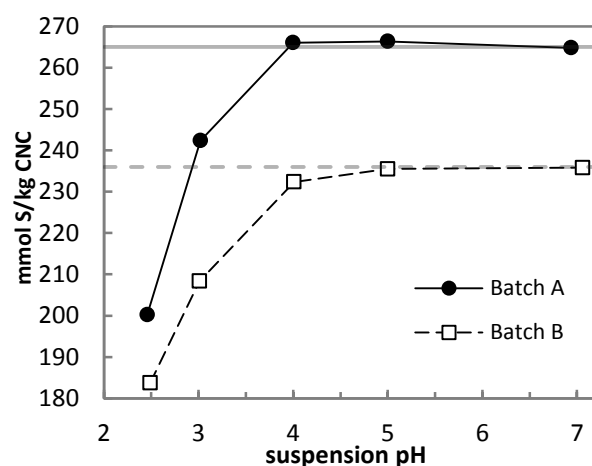


Fig 6 - Final sulfur contents of CNCs freeze-dried from suspensions of increasing pH. Grey lines indicate the initial sulfur content of each batch.

Table 6 - Sulfur content of H-CNCs during and after freeze-drying (initial suspension, 2.7 wt%).

Step	mmol S/kg CNC		%S lost
	Initial	Final	
Freezing	261	231	11.5
Freezing + sublimation	261	127	51
Storage (5 months, 23°C)	261	39	85

As expected, the pH of the original CNC suspension affects the extent of acid-catalyzed desulfation during freeze-drying. Fully protonated H-CNC suspensions have a pH of around 2 to 3 and can lose up to 50% of their sulfur groups (Table 6), while fully neutralized Na-CNCs do not undergo desulfation. Desulfation during freeze-drying is effectively eliminated in suspensions of pH 4 and above (Fig 6). This pH threshold for dried CNC desulfation is very close to the pH cut-off for dried CNC dispersibility (Beck et al. 2012). CNCs in which more than 94% of the protons have been replaced by Na^+ are thus both chemically stable and redispersible at very low moisture contents, as discussed in the previous section.

Desulfation in spray-dried CNCs

During spray-drying, droplets of aqueous CNC suspension are dried in a stream of hot ($\sim 175^\circ\text{C}$) gas to give a granular powder. Heat damage is minimal because of the very short residence times and because water evaporation cools the particles. Industrially produced spray-dried CNCs are in the water-dispersible neutral salt form (Na-CNC), which is stable to desulfation. Spray-dried H-CNCs were found to contain around 60% less sulfur than CNCs in the initial suspension. The high heat in combination with the physical drying (removal of water) is responsible for the considerable desulfation notwithstanding the very short residence time.

Effects of desulfation on CNC properties

In CNC suspensions, the removal of covalently bound surface sulfate half-ester groups decreases the surface charge density of cellulose nanocrystals and increases the ionic strength of the surrounding medium. This is only significant if it detrimentally affects desirable material

properties important to CNC applications, such as the self-assembly of cellulose nanocrystals in suspension as well as the bulk suspension behaviour and properties.

Both the lower CNC surface charge density and the higher solution ionic strength caused by desulfation will contribute to CNC agglomeration or flocculation (Dong et al. 1996; Revol et al. 1997; Araki et al. 2001; Boluk et al. 2011; Zhong et al. 2012). An extreme example of the effect of surface charge density is the CNCs produced by hydrochloric acid hydrolysis, which have only a few surface carboxylic acid groups and have a strong tendency to agglomerate and precipitate out of suspension. However, when uncharged HCl-extracted CNCs are post-sulfated to sulfate half-ester contents of 100 to 150 mmol S/kg CNC, stable suspensions are obtained (Araki et al. 1999). Sulfated CNCs are unstable (a cloudy white dispersion with precipitate forms) at sulfur contents below 65 ± 5 mmol S/kg CNC. In general, desulfation occurring during storage or short heating at temperatures lower than 90°C should not destabilize an aqueous CNC suspension.

The viscosity behaviour of CNC suspensions is influenced by the surface charge on the cellulose nanocrystals, which decreases with desulfation. Decreasing surface charge density was found to increase the volume fraction dependence of the relative viscosity of CNC suspensions and cause significant time-dependence of the suspension viscosity behaviour (Araki et al. 1999).

Desulfation of CNCs in suspension releases sulfuric acid into the surrounding medium, increasing the ionic strength and reducing the pH. In accordance with Dong and Gray (1997), we have found that as desulfation progresses, the free sulfuric acid increases the critical concentration of CNCs necessary for phase separation into isotropic and chiral nematic liquid crystal phases by screening the remaining surface charges. The volume fraction of anisotropic phase therefore decreases with decreasing sulfur content over time and then levels off.

Electrostatic screening of CNC surface charge by the sulfuric acid released by desulfation shortens the chiral nematic pitch of the liquid crystalline phase, which causes films cast from the (undialyzed) suspensions which have undergone desulfation to exhibit progressively more blue-shifted reflection wavelengths as desulfation occurs. Lower surface charge density of the purified (dialyzed) desulfated CNCs also shortens the chiral nematic pitch and hence the maximum reflection wavelength of CNC films (Revol et al. 1997; Revol et al. 1998). Finally, the magnitude of the sonication-induced chiral nematic pitch red-shift (Beck et al. 2011) also decreases as desulfation proceeds in sealed CNC suspensions which are not dialyzed prior to film casting.

Harsh conditions such as added strong acid or base (> 10 – 25 mM) combined with heat ($> 50^\circ\text{C}$) are required to chemically remove more than 10% of the sulfate half-ester groups from CNC (Table 1), meaning that CNCs are relatively resistant to desulfation under ordinary storage and handling conditions. Auto-catalyzed desulfation of CNCs is an important concern for optical applications which require controlled self-assembly in suspension, and electrical applications in which a specific surface charge

density is essential. To avoid desulfation, CNC suspensions should be stored at low temperatures in neutral form where possible. Beyond the possibility of acidic cellulose degradation, the implications of desulfation in solid forms of CNCs, such as chiral nematic self-assembled films used in optical applications, have not been determined.

Conclusions

Self-catalyzed desulfation occurs in suspensions of acidic CNCs containing proton counterions. In aqueous suspension, the desulfation rate increases with H-CNC concentration. However, dried solid H-CNCs undergo much more rapid desulfation than aqueous H-CNC suspensions. Desulfation rates in solid H-CNC rates also depend on the relative humidity of the environment, as more moisture adsorbed on the surface reduces the surface acid concentration. The desulfation kinetics for solid H-CNCs are first-order in acidic sulfate half-ester content. Lower temperatures can significantly reduce the acid-catalyzed hydrolysis reaction rate in H-CNC suspensions. Heating dispersed and solid H-CNCs above 50°C greatly accelerates the desulfation, increasing the rate constant by three or four orders of magnitude. Desulfation appears to be eliminated in aqueous CNC suspensions at pH values above 4–5; the neutral salt forms of CNCs such as Na-CNCs do not undergo significant desulfation at temperatures up to 105°C . When stored at 4°C , dilute H-CNC suspensions are relatively resistant to self-desulfation for periods of up to one year (less than 20% loss of sulfur).

Further work is needed to assess the effect of sulfate half-ester content and surface charge density on the performance of CNCs in specific applications. Specific properties of CNCs should be targeted to determine their response to desulfation. In addition, standard methods of measuring the CNC sulfate half-ester content by both conductometric titration and elemental analysis (e.g. ICP-AES), including sample preparation methods, would be very useful in determining the relationship among total sulfur content, sulfate half-ester content and surface charge in cellulose nanocrystals.

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