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Journal

ACS Sustainable Chemistry and Engineering, 4(3)

ISSN

2168-0485

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Publication Date

2016-03-07

DOI

10.1021/acssuschemeng.5b01123

Peer reviewed

Self-assembling of TEMPO Oxidized Cellulose Nanofibrils As Affected by Protonation of Surface Carboxyls and Drying Methods

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Supporting Information

ABSTRACT: Self-assembling of cellulose nanofibrils (CNFs) as affected by varying extent of protonation on C6 surface carboxyls was investigated under freeze-drying and air-drying processes. Surface carboxyls were protonated from 10.3 to 100%, all on the same TEMPO oxidized and mechanically blended CNFs with identical geometries and level of oxidation. Upon freeze-drying, all CNFs assembled into amphiphilic mass. The mostly charged CNFs assembled into the finest and most uniform fibers ($\phi = 137$ nm) that absorbed significantly more nonpolar toluene than water; whereas the fully protonated CNFs assembled extensively into porous and more thermally stable ultrathin filmlike structures that absorbed water and toluene similarly. Ultrafiltration and air-drying induced crystallization led to more thermally stable, semitransparent, and hydrophilic films that showed no affinity toward nonpolar toluene. In essence, CNFs could be tuned by varying the degree of surface carboxyl protonation, along with drying processes, to create fibrous to film morphologies, amphiphilic to hydrophilic properties, and higher thermal stability.

KEYWORDS: Surface carboxyl protonation, Cellulose nanofibrils, Amphiphilic, Hydrophobic, Self-assembly, Thermal stability, TEMPO oxidation

INTRODUCTION

The 2,2,6,6-tetramethylpyperidine-1-oxyl (TEMPO) mediated oxidation of cellulose breaks interfibril hydrogen bonds and converts C6 primary hydroxyls to carboxyls and/or carboxylates depending on the conditions.^{1–3} TEMPO-mediated oxidation is among the most investigated defibrillation methods to liberate cellulose nanofibrils (CNFs) and has shown to reduce energy needed for defibrillating cellulose by at least 2 orders of magnitude.³ The defibrillation efficiency can be further aided by mild to moderate mechanical post-treatments that have included magnetic stirring,⁴ blending,^{1,5} and brief sonication^{6,7} to produce CNFs with varied surface carboxyls/carboxylates and geometries. Dissociation of surface carboxylates provides electrostatic repulsion to disperse CNFs as homogeneous aqueous suspensions. While replacing Na^+ counterions with other monovalent (Ag^+), divalent (Ca^{2+} , Zn^{2+} , Cu^{2+}), and trivalent (Al^{3+} , Fe^{3+}) cations could cause hydrogelation via chelation with CNF carboxylate anions^{8,9} and protonation of sodium carboxylate to carboxylic acids could induce gelation to pH-responsive CNF hydrogels.^{10–12} Fully protonated CNFs also have shown to exhibit much better dispersibility in polar aprotic solvents, such as *N,N*-dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), 1,3-dimethyl-2-imidazolidinone (DMI), and 1-methyl-2-pyrrolidone (NMP).¹³

Protonation of surface carboxyls has also shown to affect the properties of solidified CNFs. Air-dried cast films from fully

protonated CNFs showed higher oxygen and hydrogen permeability,^{14,15} more resistant to cellulase and soil micro-organisms degradation and less water absorbing.¹⁶ The higher gas permeability of fully protonated CNF film was thought to be due to the larger free volume generated from interfibrillar hydrogen bonding,¹⁴ but such speculation has not been confirmed by physical evidence. Thus, how protonation of surface carboxyls on CNFs affects their association into solids during drying has yet to be clearly delineated.

Drying nanocellulose into solids is critical to its applications. Various drying methods have led to different solid forms, including air-dried films,^{17,18} spray-dried powders,¹⁹ freeze-dried fibers^{1,20–22} and aerogels,^{23–25} supercritically dried porous paper,²⁶ and aerogel.²⁷ The redispersibility of these respective dried solids in water^{21,24–26} and organic solvent²¹ have been reported. The solid morphologies from freeze-drying have been shown to be dependent on CNF surface carboxylate contents¹ and concentrations,^{20,23} a dispersing medium,^{20,28} and freezing-temperatures.^{20,24} While highly surface oxidized CNFs associate with each other extensively into more heterogeneous forms of thicker fibers of over 500 nm widths to micrometer wide films,¹ more uniform and finer fibers could only be assembled from CNFs with low surface carboxyl

Received: September 18, 2015

Revised: December 23, 2015

Published: January 26, 2016

content of 0.59 mmol/g cellulose (125 nm),¹ at extremely low concentration of 0.01 wt % (236 nm wide),²⁰ or from codispersing in a tert-butanol medium (33–43 nm).²³ As varied surface carboxylation was generated by different extents of oxidation that also affected CNF dimensions, both may have influenced the resulting morphologies. Therefore, the effects of surface charge or protonation of surface carboxyls on CNF properties and their solid structure from drying have not been systematically established.

This study was designed to understand how the extent of CNF surface carboxyl protonation affected their aqueous suspension properties and self-assembled structures using CNFs defibrillated from rice straw cellulose by one coupled TEMPO oxidation and mechanical blending condition,³ such that all having the same geometric dimensions and level of surface oxidation. The CNF surface C6 carboxyls were protonated with acid or deprotonated with base, respectively, to generate CNFs containing a range of surface carboxylic acid (COOH) and sodium carboxylate (COO[−]Na⁺), all with the same total carboxyls (COOH/COO[−]Na⁺). The suspension properties as affected by varying protonation of CNF surface carboxyls was studied to establish the charge repulsion and hydrogen-bonding effects. Drying induced assembling of CNFs with varied surface carboxyl protonation was conducted by either rapid liquid nitrogen freezing followed by freeze-drying or ultrafiltration followed by air-drying. The structures and morphology as well as crystallinity, liquid absorption and thermal stabilities of these assembled solids were characterized to relate to the surface protonation effects and the assembling mechanisms of CNFs.

EXPERIMENTAL SECTION

Materials. Pure cellulose was isolated from rice straw to 36% yield by a three-step 2:1 toluene/ethanol extraction, acidified NaClO₂ (1.4%, pH 3–4, 70 °C, 6 h) and KOH (5%, 70 °C, 2 h) isolation process reported previously.²⁹ Hydrochloric acid (HCl, 1 N, Certified, Fisher Scientific), sodium hydroxide (NaOH, 1 N, Certified, Fisher Scientific), sodium hypochlorite (NaClO, reagent grade, Sigma-Aldrich), 2,2,6,6-tetramethylpiperidine (TEMPO, 99.9%, Sigma-Aldrich), sodium bromide (NaBr, BioXtra, 99.6%, Sigma-Aldrich), and toluene (Certified ACS, Fisher Scientific) were used as received. All water used was purified by Milli-Q plus water purification system (Millipore Corporate, Billerica, MA).

CNFs Isolation and Tuning Surface Carboxyl Protonation. Rice straw cellulose was defibrillated into cellulose nanofibrils (CNFs) via TEMPO mediated oxidation employing 5 mmol/g NaClO/cellulose at pH 10, then neutralized to pH 7 by adding 0.5 M NaOH, followed by mechanical blending (Vitamix 5200) at 37 000 rpm for 30 min.¹ The surface carboxyl protonation was tuned by adding specific quantities of HCl (0.1 M) or NaOH (0.1 M) to 150 mL 0.13 wt % CNF suspension under vigorous stirring for at least 30 min to prevent aggregation. The suspension was then dialyzed against water for 2 days to remove excess acid or base. The obtained CNFs with increased surface carboxyl protonation were designated as CNF1 (4 mL NaOH added), CNF2 (as is), CNF3 (1 mL HCl added), and CNF4 (6 mL HCl added).

Assembly of CNFs via Freeze-Drying and Ultrafiltration. Aqueous 0.1 wt % CNF suspensions were dried in two different ways: rapid freezing then freeze-dried and ultrafiltration then air-dried. Aq. CNF suspensions (20 mL) were rapidly frozen in liquid nitrogen (−196 °C) for 10 min then lyophilized (−50 °C, 0.05 mbar) for 2 days in a freeze-drier (FreeZone 1.0L Benchtop Freeze-Dry System, Labconco, Kansas City, MO). Aq. CNF suspensions (60 mL) were filtered through nylon membrane (200 nm pore size, Millipore, Billerica, MA), allowed to air-dry under ambient conditions, then

rewetted to be detached, and further dried in between two membranes at room temperature.

Characterizations. The pH and conductivity of the CNF suspensions were measured using OAKTON pH/Con 510 series meter with pH and conductivity probes, respectively. The surface carboxyls on CNFs were determined by conductometric titration of 0.1 wt % CNF suspension with 0.01 M NaOH solution. The surface carboxyl content (σ) was calculated from the equation

$$\sigma = \frac{cv}{m} \quad (1)$$

where c is the NaOH concentration (M), m is the total mass of CNF in the suspension (g), and V is the amount of NaOH consumed by the carboxyls (mL). The optical transmittance of 0.1 wt % CNF suspensions was recorded from 300 to 800 nm using Evolution 600 UV–vis spectrophotometer (Thermo Scientific) in quartz cuvette. CNFs (10 μ L, 0.0005 wt %) were deposited on freshly cleaved mica, dried in air, and scanned using atomic force microscope (Asylum-Research MFP-3D, Santa Barbara, CA) equipped with OMCL-AC160TS standard silicon probes under tapping mode at 1 Hz scan rate and 512 $\times$ 512 pixel resolution.

Freeze-dried CNFs were pressed in KBr pellets (1:100, w/w) and collected using a Thermo Nicolet 6700 spectrometer at ambient conditions and 4 cm^{−1} resolution from an accumulation of 128 scans over the regions of 4000–400 cm^{−1}. Freeze-dried CNFs were mounted with conductive carbon tape, sputter coated with gold, and imaged by a field emission scanning electron microscope (FE-SEM) (XL 30-SFEG, FEI/Philips, USA) at a 5 mm working distance and 5 kV accelerating voltage. The diameters of freeze-dried CNFs were calculated from measurements of over 100 individual fibers using an image analyzer (ImageJ, NIH, USA). Elemental analysis of freeze-dried CNFs was conducted in triplicate using EDS (EDAX, AMETEK, Inc.) on the scanning electron microscope at 500 $\times$ magnification with 10 kV accelerating voltage and 5 mm working distance, and the average value and standard deviation were reported. The optical transmittance of air-dried CNF films was recorded from 350 to 800 nm using Evolution 600 UV–vis spectrophotometer.

Both freeze- and air-dried CNFs were scanned on a Scintag XDS 2000 powder diffractometer using a Ni-filtered Cu K α radiation (λ = 1.5406 Å) at an anode voltage of 45 kV and a current of 40 mA from 5° to 40° at a scan rate of 2°/min. Freeze-dried samples were compressed between two glass slides into flat sheets with around 1 mm thickness, whereas air-dried films were scanned as is. Crystallinity index (CrI) was calculated from the intensity of the 200 peak (I_{200} , 2θ = 22.6°) and the intensity minimum between the peaks at 200 and 110 (I_{am} , 2θ = 18.7°) by using the empirical equation³⁰

$$\text{CrI} = \frac{I_{200} - I_{\text{am}}}{I_{200}} \times 100 \quad (2)$$

The thermal behaviors of freeze- and air-dried CNFs were investigated using differential scanning calorimetry (DSC-60, Shimadzu, Japan) and thermogravimetric analyzer (TGA-50, Shimadzu, Japan) at a heating rate of 10 °C/min from 25 to 500 °C under purging N₂ (50 mL/min).

The morphologies of assembled fibers and films were scanned using atomic force microscope as previously described. Freeze-dried CNF1 was dispersed in water and then dried onto freshly peeled mica, and the air-dried films were taped onto glass slides. The root square mean (RSM) roughness of air-dried film was calculated based on scanning over a 5 μ m $\times$ 5 μ m area on the AFM images using MFP3D 090909 + 1409 plugin in IGOR Pro 6.21.

The ability of the freeze- and air-dried CNFs to absorb polar water and nonpolar toluene was measured. Air-dried CNFs were weighed (w_{dry}) and put into liquid for 30 min, and the wet films were taken out and all surface liquid were blotted with Kimwipes and then weighed again (w_{wet}). Freeze-dried CNFs were dispersed in liquids (5/20 mg/mL), allowed to sit for 30 min, and then centrifuged at 5000 rpm for 30 min. The wet precipitate were weighed (w_{wet}) and dried in oven to

Figure 1. Characterization of CNFs in aqueous (a, c) and freeze-dried (b, d) states: (a) conductometric titration curves, (b) EDS, (c) UV–vis transmittance, (d) FTIR, and (e) pH, conductivity, surface COOH, and Na content.

weigh again (w_{dry}). The liquid absorption ratios (LARs) were determined as

$$\text{LAR} = \frac{(w_{\text{wet}} - w_{\text{dry}})}{w_{\text{dry}}} \quad (3)$$

RESULTS AND DISCUSSION

Surface Characteristics. TEMPO-mediated oxidation of cellulose oxidizes the primary C6 hydroxyls in the amorphous regions and on the crystallite surfaces into carboxylic acids, which were immediately ionized into sodium carboxylates at pH 10. Neutralization to pH 7 at the end of reaction protonated approximately 14% sodium carboxylates into carboxylic acid,¹ leaving the majority negatively charged to facilitate the subsequent mechanical defibrillation into homogeneous aqueous CNF2 suspension. The predominantly charged sodium carboxylated CNF2 was deprotonated with NaOH or protonated with HCl to respective CNF1 or CNF3 and CNF4 with varied extents of ionized sodium carboxylate (COO^-Na^+) and protonated carboxylic acid (COOH) at the same total carboxyl ($\text{COO}^-\text{Na}^+/\text{COOH}$) content. Titrating aqueous suspensions with NaOH showed unchanged conductivity for all four CNFs, followed by similarly sharp increases (Figure 1a). The plateau region represented neutralization of CNF surface carboxylic acids whereas the abrupt increases reflected accumulated NaOH beyond neutralization. The surface COOH contents were calculated from the plateau regions to be 0.12, 0.28, 0.72, and 1.16 mmol/g for CNF1, CNF2, CNF3, and CNF4, respectively, showing increasing protonation (Figure 1e). EDS spectra showed prominent C and O peaks that are typical of cellulose (Figure 1b). A very small Na peak at

around 1.02 keV was observed for CNF1, became barely observable for CNF2 and CNF3, then completely disappeared for CNF4, showing 1.84, 1.36, 1.01, and 0 at % Na for CNF1, CNF2, CNF3, and CNF4, respectively (Figure 1e). The absence of Na in CNF4 confirms the complete protonation of sodium carboxylates into carboxylic acids, thus its 1.16 mmol/g COOH content represents the total carboxyl content for these TEMPO-oxidized CNFs. The COO^-Na^+ contents for CNF1, CNF2, CNF3 and CNF4 could, therefore, be derived by deducting individual carboxylic acid contents from the same total carboxyl to 1.02, 0.88, 0.44, and 0 mmol/g or 10.3, 24.1, 62.1, and 100% protonated, respectively.

All four aqueous CNF suspensions were acidic and conductive with CNF1 having the highest pH and conductivity that were expected with its highest COO^-Na^+ content of nearly 90%. The 5.6 pH value of CNF 1 is close to that of DI water in air (pH 5.5 due to the dissolution of CO_2), indicating nearly no proton dissociation; and the high conductivity of 45 $\mu\text{S}/\text{cm}$ was consistent with highly dissociated COO^-Na^+ ions. Both pH and conductivity decreased with increasing protonation, lowering to respective 4.1 and 10 $\mu\text{S}/\text{cm}$ for the most protonated CNF4. As carboxylic acid on CNFs is weak acid with pK_a around 3.6–3.7,^{14,15} not all surface carboxylic acid could completely dissociate at pH 4–5, therefore lower ion concentrations and conductivity are expected for the more protonated CNFs.

The optical transmittance of aq 0.1 wt % CNF1, CNF2, CNF3, and CNF4 suspensions were 79–96, 74–96, 72–95, and 64–90% from 300 to 800 nm wavelengths, respectively (Figure 1c). The reduced transmittance with increasing protonation is indicative of greater association of the less ionized CNFs. Correspondingly, AFM images of 0.0001%

Figure 2. SEM images of freeze-dried CNF1 (a, e), CNF2 (b, f), CNF3 (c, g), and CNF4 (d, h).

CNF4 showed several large aggregates where fewer were observed in other CNFs (Figure S1, [Supporting Information](#)), consistent with optical transmittance data. However, individual CNFs could still be clearly observed for all CNFs under AFM, signifying mostly CNFs were well-dispersed at this very dilute concentration.

FTIR spectra of all four CNFs showed typical cellulose characteristic peaks at 3394 cm^{-1} (O–H stretching), 2911 cm^{-1} (C–H stretching), 1417 cm^{-1} (H–C–H and O–C–H in-plane deformation), 1371 cm^{-1} (C–H deformation vibration), 1317 cm^{-1} (H–C–H wagging vibration), 1201 cm^{-1} (C–O–H in-plane deformation), 1163 cm^{-1} (C–O–C asymmetric bridge stretching of the β -glycosidic linkage), 1057 cm^{-1} (C–O–C pyranose ring skeletal vibration), and 897 cm^{-1} ($\text{C}_1\text{--O--C}_4$ deformation of the β -glycosidic linkage) (Figure 1d). The major spectral differences among the four CNFs reside at 1728 and 1612 cm^{-1} , corresponding to the respective carbonyl stretchings in COOH and COO^-Na^+ . A sharp 1612 cm^{-1} peak and a small shoulder at 1728 cm^{-1} of CNF1 confirmed the dominance of COO^-Na^+ , also consistent with the highest conductivity, pH and Na content discussed earlier. The 1728 cm^{-1} peak intensified while the 1612 cm^{-1} diminished from CNF1 to CNF4, clearly indicative of the progressive COO^-Na^+ to COOH conversion. The disappearance of the 1612 cm^{-1} peak in CNF4 further confirmed the complete COO^-Na^+ to COOH conversion, with the O–H deformation peak of absorbed water reappearing at 1632 cm^{-1} .

Self-Assembled CNFs via Freeze-Drying. The self-assembling behaviors of CNFs were first observed by rapid freezing in liquid nitrogen, followed by freeze-drying. The rapidly frozen and lyophilized solids from all four CNFs were fluffy and appeared similarly white, indicative of substantial self-assembling into macroscopic structures, losing the original nanoscale dimensions. The self-assembling behaviors of all CNFs was observed at the same $0.1\text{ wt } \%$ to rule out of the concentration effect on the self-assembled morphologies as observed previously,²⁰ to focus on effects of the varied protonation of surface carboxyls. The nearly 90% carboxylated and least protonated CNF1 assembled exclusively into the finest fibers, with $137 (\pm 57)\text{ nm}$ average diameter (ϕ). The considerably charged CNF2 with 76% carboxylated surfaces assembled into thicker 300 nm to several μm wide ribbons, while some ca. 100 nm wide fibers were also present. The much less 38% carboxylated CNF3 and the completely protonated CNF4 assembled into yet thicker several μm wide ribbons and thin film pieces, with much fewer submicron or ca. 100 nm wide fibers. The finest fibers assembled from CNF1 had very

close ϕ to those assembled from TEMPO oxidized CNFs with low surface carboxyl content of 0.59 mmol/g (125 nm),¹ uncharged CNFs from high speed blending (153 nm)³¹ as well as aqueous counter collisions treatment (136 nm).³² Interestingly, finer and more uniform submicron sized fibers were more favorably assembled from CNFs that were either unoxidized, with low surface carboxyl groups, or with highly dissociated carboxyls. One common structural feature among these CNFs is the absence or minimal presence of carboxylic acid, suggesting extensive CNF self-assembling or inter-CNF association via hydrogen bonding to be dependent on sufficient carboxylic acid on CNF surfaces.

AFM images of freeze-dried CNF1 redispersed in water showed both submicron and nanofibers (Figure 3a), with their height profiles similar to the 137 nm ϕ observed by SEM (Figure 2), indicating the submicron assembled fibrils from CNF1 were tenaciously hydrogen bonded together and could not be disrupted with gentle shaking in water. The compact structure of the assembled fibers was clearly evident from the amplitude image with smooth surfaces and no discernible individual CNFs (Figure 3b), indicating perfectly aligned and tenaciously bound CNF1. The massively self-assembled CNF4

Figure 3. AFM height (a) and amplitude (b) images of freeze-dried CNF1 after redispersing in water and higher magnification SEM images of freeze-dried CNF4 (c, d). (a inset) Height profile along the red line.

Figure 4. N₂ adsorption–desorption isotherms (a) and pore size distribution (b) of CNF1–4. (a inset) Specific surface areas and BJH pore volume.

Figure 5. Ultrafiltered and air-dried films: (a) UV–vis transmittance spectra; AFM amplitudes images of (b) CNF1, (c) CNF2, (d) CNF3, and (e) CNF4.

contained mostly porous film structures upon closer inspection, with numerous 5–20 nm wide pores and a few individual fibers protruding from the surface (Figure 3c). Similar to fibers assembled from CNF1, the self-assembled submicron fibers from CNF4 also appeared solid with no evidence of porous defects (Figure 3d). Thus, all self-assembled structures from the varied protonated CNFs remained intact when redispersed in water.

The nitrogen adsorption–desorption isotherms for all freeze-dried CNFs showed nearly reversible adsorption and desorption loops (Figure 4a) or type II isotherms that are typical for nonporous or macroporous materials. The BET surface areas were 32.9, 35.9, and 38.3 m²/g, increasing slightly with increasingly protonated CNF1, CNF2 and CNF3, respectively, and doubled to 65.7 m²/g for the fully protonated CNF4. Similarly, their pore volumes also increased slightly, i.e., 0.12, 0.13, and 0.14 cm³/g for CNF1, CNF2, and CNF3, respectively, and nearly doubled to 0.21 cm³/g for CNF4. Pore size distributions showed all four to contain both meso- and macropores with those in CNF1–CNF3 to be 20–100 nm wide (Figure 4b); whereas significantly more pores were found in CNF4, in particular the smaller 2–30 nm wide mesopores, consistent with SEM observation (Figure 3c, d).

The distinctions in morphologies and pore characteristics among these assembled structures are clearly associated with the varied degrees of protonation since all CNFs have the same geometric dimensions and total surface carboxyls. The most negatively charged CNF1 had the highest electrostatic repulsion to minimize interfibrillar association to the least assembled and thinnest fibers. Increasing protonation lowers repulsion among the less charged CNFs, permitting more substantial self-assembly into thicker ribbons and even wider, but porous thin films. Besides, the surface carboxylic acid

groups could instantaneously hydrogen bond interfibrillarly, negating further interfibril rearrangement to form more porous structure within the massively self-assembled thin films. Therefore, CNFs may assemble into nanofibrous to porous film-like structures during rapid freezing and freeze-drying, and the extent of assembling could be regulated facilely by tuning the extent of surface carboxyl protonation.

Previous work has shown that films casted from TEMPO-oxidized CNFs with only carboxylic acid groups had significantly higher O₂ and H₂ permeability than those from CNF with sodium carboxylates.^{14,15} The higher gas permeability was only hypothesized to be from the larger free volume formed by interfibrillar hydrogen bonds,¹⁴ without being substantiated by specific data. The SEM and BET data presented here provide the first physical evidence that highly porous structure with high specific surface was more favorably assembled from fully protonated CNFs with all surface carboxyls in the form of carboxylic acid.

Self-Assembled CNFs via Ultrafiltration and Air-Drying. Ultrafiltration of aqueous CNFs followed by air-drying generated semitransparent films, in contrast to the white fluffy mass from rapid freezing and slow freeze-drying. The optical transmittance of air-dried films of ca. 40–50 μm thickness was 53–81, 47–81, 30–73, and 9–38% in the 350 to 800 nm wavelength range for CNF1, CNF2, CNF3, and CNF4, respectively (Figure 5a). Film transparency lowered slightly for CNF3 and most distinctively for CNF4, indicative of higher extent of CNF assembling for the CNF4 film. The much lower optical transmittance of the air-dried films than their respective 0.1% aqueous suspensions (Figure 1b) was another indication of CNF aggregation. The film surfaces were irregular as profiled by AFM (Figure 5b–e), but without any trace of individual or bundled CNFs nor observable pores (Figure 5b–d), indicative

Figure 6. Representative XRD curves of air-dried and freeze-dried CNF4 (a). Crystallinity index (b) of CNF1 to CNF4.

Table 1. Liquid Absorption Ratios (LARs) of Freeze- and Air-Dried CNFs

drying methods/liquids	liquid absorption ratios (g/g)			
	CNF1	CNF2	CNF3	CNF4
air-dried/water ^a	2.60 ± 0.07	1.84 ± 0.05	1.24 ± 0.04	0.61 ± 0.05
air-dried/toluene	0	0	0	0
freeze-dried/water	74.4	65.6	60.5	95.0
freeze-dried/toluene	136.1	145.2	101.5	102.6

^aAveraged values from three measurements.

of tightly packed CNFs and consistent with the significantly decreased optical transmittance. The root-mean-square (RMS) roughness of these films was 18.1, 23.4, 18.2, and 14.3 nm for CNF1, CNF2, CNF3, and CNF4, respectively, showing no particular trend and indicating film surface roughness to be independent of CNF surface charges.

Comparisons of Freeze- and Air-Dried CNF Characteristics. The XRD spectra of both freeze- and air-dried CNFs showed characteristic monoclinic cellulose Iβ 110, 110, and 200 crystallographic lattice peaks at 14.7, 16.8 and 22.7°, respectively (Figure 6a). The freeze-dried CNFs had CrIs ranging from 66.3 to 74.5%, 10–20% lower than the air-dried counterparts with very similar 79.3–81.6% CrIs (Figure 6b). The low CPS values and high noises of the freeze-dried CNF were attributed to its less compacted porous structure. The higher CrIs of freeze-dried CNF1 and CNF4 suggest that CNFs with more homogeneous surface chemistries, i.e., either mostly highly carboxylated CNF1 or the fully protonated CNF4, may assemble into more crystalline structure. In the case of air-dried samples, strong vacuum pulling forces in ultrafiltration also seem to dominate over surface charge effects among the four differently protonated CNFs to enhance internanofibril compaction and crystallization. In essence, the XRD results suggest that the CrIs derived from freeze-dried structure may be closer to the actual crystallinity of CNFs, whereas those of air-dried films likely overestimate due to ultrafiltration force induced crystallization.

The ability of air-dried and freeze-dried CNFs to absorb polar (water) and nonpolar (toluene) liquids was determined by their liquid absorption ratios (LARs) (Table 1). The air-dried films from CNF1, CNF2, CNF3, CNF4 absorbed 2.6, 1.84, 1.24, 0.61 g/g water, respectively, decreasing with increasing protonation. None showed any change in thickness or planar dimension, indicating internal water absorption that suggested the presence of interfibrillar pores within the films. That CNF1 film absorbed 4 times more water than CNF4 is consistent with the more ionized but least hydrogen bonded structure of CNF1. Most significantly, none of air-dried films absorbed any nonpolar toluene, showing highly polar and

hydrophilic nature. It is worth of noting that although these ultrafiltered and air-dried solids appear filmlike, water absorption of those from CNF2 and CNF3 are similar to common woven cotton fabrics.

In contrast to the highly compact structure and hydrophilic nature of air-dried films, freeze-dried fluffy fibrous mass absorbed not only 60–90 g/g of water, i.e., 29 (CNF1) to 155 (CNF4) times, far more than the air-dried counterparts, but also significant toluene, i.e., 101–145 g/g, clearly indicative of their highly porous and superabsorbent structures and, most significantly, amphiphilicity. Furthermore, freeze-dried CNFs are more hydrophobic than hydrophilic, absorbing 8 (CNF4) to 80% (CNF1) more toluene than water. Due to the density difference, the fibrous mass from freeze-dried CNF 1, CNF2, and CNF3 absorbed about two times in volume of toluene than water, significantly more hydrophobic.

That CNFs self-assemble into structures with dramatically distinct affinity to polar and nonpolar liquids is very intriguing. Upon rapid freezing in liquid nitrogen at −196 °C, repulsion among negative charge hydrophilic facets of the decreasingly charged CNF1 to CNF3 prevented interfibril association and hydrogen bonding along the 110 and 110 crystalline plane surfaces, where association and self-assembling of CNFs may occur predominantly among the hydrophobic 200 facets, imbedding more of the hydrophobic surfaces while exposing more of the hydrophilic surfaces. The amphiphilicity and higher nonpolar liquid absorption of those from CNF1, CNF2, and CNF3 suggest that not only hydrophilic and hydrophobic surfaces are available, but the relative quantities and/or accessibility of hydrophobic areas may also be higher. Whereas the uncharged CNF4 may associate and assemble along both hydrophilic and hydrophobic planes randomly, leading to similar amount of water and toluene absorption.

Our previous work has shown extensive association of 0.3% CNF2 by slow freezing (−20 °C for 15 h) then freeze-drying (−50 °C, 0.05 mbar, 2 days) could produce amphiphilic aerogels that absorb 210 and 144 g/g of polar water and nonpolar decane.²³ Based on their densities, the volume of water absorbed was 7% higher than decane, showing aerogels to

be slight more hydrophilic. While liquid absorbed in aerogel primarily reside in the micrometer scale pores surrounded by assembled film/wall, different than the capillary liquid in between the fibrous mass from CNF2 here, the over two time more toluene (145 g/g or 168 cm³/g) absorbed than water (65.6 g/g) of the latter clearly show the extent of hydrophobic versus hydrophilic surfaces to be considerably more from rapid (−196 °C) than slow (−20 °C) freezing where the charge effect seems to be far less. In fact, the near balanced absorption of nonpolar and polar liquids was observed on the fibrous mass rapidly frozen from the uncharged CNF4 and the aerogel slowly frozen from the charged CNF2, suggesting that charge effects on CNF self-assembling could only occur under rapid freezing condition.

Pure rice straw cellulose lost 2.5% absorbed moisture at below 150 °C, remained thermally stable at up to 280 °C, then lost 90% mass rapidly up to 380 °C, showing a corresponding decomposition exotherm at 343 °C and a charring exotherm at 447 °C, leaving 3.3% charred residue at 500 °C (Figure 7).

Figure 7. TGA and DSC curves of rice straw cellulose and TEMPO oxidized cellulose before and after blending.

TEMPO oxidation alone increased hydrophilicity as shown by the 8.8% moisture loss, but lowered the onset decomposition

temperature to 200 °C and a corresponding exotherm around 210–330 °C, approximately 80 °C lower than that of pure cellulose. Such severely deteriorated thermal stability may be linked to the direct solid-to-gas phase transitions from decarboxylation of surface carboxyl groups.^{1,31,33} A second exotherm appeared over a broader range of temperature than that for pure cellulose, leaving a much higher 16.9% char for TEMPO oxidized cellulose, 5.1 times of pure cellulose. Mechanical blending did not alter the onset decomposition temperature, but enlarged the first exotherm and shifted the second exotherm to a much lower 400 °C and nearly halved the char to 8.2%, possibly due to the increased specific surface from mechanical defibrillation.

All dried CNFs were hygroscopic, evaporating 6.3–9.7% moisture and decomposed at lower onset temperatures of ca. 200 to 350 °C, but leaving substantially more chars from the air-dried (30–32%) than the freeze-dried (8.1–13.8%) ones (Figure 8). All air-dried CNFs lost ca. 40% mass in two stages near 258–273 ($T_{\max 1}$) and 312–337 °C ($T_{\max 2}$) (Figure 8b), corresponding to degradation of the respective carboxylated surface chains (stage I) and unmodified crystalline cores (stage II).^{33,34} CNF1 and CNF2 showed nearly identical mass loss patterns (Figure S2, Supporting Information; inset in Figure 8a) due to their closer surface charges. Slightly lower weight loss rate at stage I and increased $T_{\max 2}$ at stage II were observed for air-dried CNF3 and CNF4, possibly due to their more protonated and hydrogen bonded structures. Freeze-dried CNF1–3 exhibited only one degradation peaking at 270–292 °C, just above decomposition stage I temperatures of the air-dried ones, with much steeper weight losses (Figure 8c), suggesting simultaneous decomposition of the crystalline cores and the carboxylated surfaces, possibly due to the much shorter heat diffusion paths of the ultrafine fibers. The rapid and simultaneous decomposition for freeze-dried CNFs is also supported by their much larger exotherms at 200–350 °C (1.79 kJ/g) than the air-dried counterpart (0.34 kJ/g) (Figure S3a, Supporting Information) which decomposed much slower and

Figure 8. TGA (a, c) and DTGA (b, d) curves of air-dried (a, b) and freeze-dried (c, d) CNFs (color codings apply to all and CNF1 was omitted from a and b due to great overlapping with CNF2. (insets in a and c) Maximum degradation temperature ($T_{\max}$) and char residues at 500 °C.

released less heat. However, freeze-dried CNF4 showed two degradation peaks at 269 and 330 °C, close to the respective stage I and II temperatures, with the latter dominant, as well as similar DTGA and DSC patterns to its air-dried counterparts (Figure S3b, Supporting Information). This similar degradation pattern for both freeze-dried and air-dried fully protonated CNF4 could be due to the preferable removal of carboxylic acid groups by direct solid-to-gas phase transition at lower temperatures, leaving more pristine crystalline cellulose structures that degrade at higher temperatures.

The crystalline structure and liquid absorption data provide direct evidence that internanofibril compaction and recrystallization from ultrafiltration forces and slow air drying lead to less surfaces being exposed to heat and improve thermal stability. Besides, fully protonated TEMPO-oxidized CNF4 with only C6 carboxylic acid groups was more thermally stable and remain similarly stable even though dried into different morphological structures by the two drying methods.

CONCLUSION

Self-assembling of TEMPO oxidized and mechanically blended CNFs with C6 surface carboxyls protonated to 10.3, 24.1, 62.1, and 100% under different drying processes were systematically investigated. Self-assembling of CNFs by rapid freezing in liquid nitrogen and freeze-drying was significantly dependent on the protonation of surface carboxyls. The nearly 90% charged and least protonated CNF1 assembled exclusively into the most homogeneous submicron nonporous fibers ($\phi = 137$ nm, 32.9 m²/g specific surface, 0.12 cm³/g pore volume). With increasingly protonation, the less surface charged CNF2 and CNF3 assembled more extensively into thicker fibers and wide ribbons while the fully protonated CNF4 assembled mostly into several micrometer wide ribbons and mesoporous thin films (5–20 nm pore sizes, 65.7 m²/g specific surface, 0.21 cm³/g pore volume). All freeze-dried CNFs were amphiphilic and superabsorbent toward both water (61–95 g/g) and toluene (102–145 g/g or 117–168 cm³/g). The higher toluene absorption could be due to the preferential assembly of charged CNFs through the hydrophobic 200 facets, where nonpolar toluene could more freely penetrate. Besides, among all freeze-dried CNFs, higher thermal stability is observed for the most protonated CNF4, which was credited to the loss of carboxylic acid groups at lower temperatures below the degradation temperatures of the crystalline cellulose cores. Ultrafiltration and air-drying, on the other hand, compacted CNFs and induced inter-CNF crystallization into semitransparent, hydrophilic and more thermally stable films that absorbed no nonpolar liquids and substantially less water (2.6–0.6 g/g) than the freeze-dried counterparts. While the surface charge protonation of CNFs significantly influenced the assembled morphologies by freeze-drying and their properties, their effects were superseded by ultrafiltration and air-drying. Most intriguingly, amphiphilicity or hydrophobicity of the assembled solids could only be achieved with rapid freezing induced self-assembling, in the fibrous structures as reported here or in the forms of aerogels reported earlier.²³

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b01123.

AFM height images of CNF1–4. TGA and DTGA curves of air-dried CNF1 and CNF2. DSC curves of air- and freeze-dried CNF2 and CNF4. TGA and DTGA curves of freeze-dried CNF2 at –20 and –196 °C (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support for this research from the California Rice Research Board (Project RU-9) is greatly appreciated.

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