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Transparent, water-resistant hybrid nanopaper by physical crosslinking with chitosan

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ABSTRACT. One of the major, but often overlooked, challenges towards high end applications of nanocelluloses is to maintain their mechanical stability under hydrated conditions. As such, permanent covalent crosslinking or surface hydrophobization are viable solutions provided that

neither processability nor interfibrillar bonding is compromised. Here we show an alternative based on physical crosslinking of nanofibrillated cellulose (NFC, also denoted as microfibrillated cellulose, MFC, and cellulose nanofibers, CNF) with chitosan for the preparation of transparent films. Transparency (~ 80 % throughout the visible spectrum) is achieved by suppressing aggregation and carefully controlling the mixing conditions: Chitosan dissolves in aqueous medium at low pH and under these conditions the NFC/chitosan mixtures form easily processable hydrogels. A simple change in the environmental conditions (i.e. an increase of pH) reduces hydration of chitosan promoting multivalent physical interactions between NFC and chitosan over those with water, resulting effectively in crosslinking. Films of NFC/chitosan 80/20 w/w show excellent mechanical properties in the wet state, with a tensile modulus of 4 and 14 GPa at low (0.5 %) and large (16 %) strains, respectively and an ultimate strength of 100 MPa (with corresponding maximum strain of 28 %). Remarkably, a strength of 200 MPa (with maximum strain of 8%) is measured at 50 % air relative humidity. We expect that the proposed, simple concept opens new pathways toward NFC-based material utilization in wet or humid conditions, a challenge that has remained unresolved.

Introduction

Nanofibrillated cellulose is a nanoscale fibrillar material (schematic representation in Figure 1a) that has drawn significant attention in recent years due to its excellent mechanical properties based on its native hydrogen bonded internal crystalline structure, renewability, availability, and biocompatibility.^{1,2} It allows strong films, leading to several potential applications including structural materials and gas barriers in packaging, templates for functional materials, and substrates for transparent electronics and devices.^{3,4,5,6,7,8,9,10,11} The strength of the junctions between the nanofibers forming the disordered networks is central to transfer the high mechanical properties of

the individual nanofibers up to the macroscopic material. Such interactions can be subtle, involving hydrogen bonding and other interactions. Upon hydration by elevated ambient humidity or contact with water, the junctions between the NFC fibers weaken and the resulting material suffers from loss of mechanical properties since water molecules penetrate into the network and compete with, for example, the interfibrillar hydrogen bonds.^{12, 13} This can become a serious issue in NFC applications as it is inherently highly hygroscopic. The mechanical properties in the wet state can be improved by covalent crosslinking, either directly between the NFC nanofibers or mediated by crosslinkable polymer matrices. In the first case, simply heating can lead to the formation of ester bonds between carboxyl and hydroxyl groups in cellulose, as first reported for papermaking fibers, an effect that can be augmented in the presence of poly-acids to yield a high degree of wet-strength.^{14, 15} Such observations can be translated to nanocellulose, as demonstrated more recently.¹⁶ In the second case of crosslinkable polymer matrices initial efforts with MFC/NFC included phenolic resins, melamine formaldehyde and epoxy, among others.^{17, 18, 19} However, covalent crosslinks bind the structures permanently and therefore hamper post-processing or end-use. Covalent crosslinking can also lead to loss of toughness as is commonly seen in rigid and brittle covalently crosslinked hydrogels and thermoset polymers.^{20, 21} Also, most of the methods demand multiple steps, which limits scalability.

A method for crosslinking by changing the environmental parameters such as temperature or pH could offer the benefit of inducing crosslinks that are reversible after processing, like has been reported for thermoreversible hydrogels.²² This could potentially be of practical importance also in fiber spinning of NFC, inspired by silk, as the coagulated NFC gel is very weak in the wet state before drying.^{23, 24, 25}

Figure 1. a) Molecular structure of cellulose and illustration of cellulose chains forming cellulose nanofibrils with crystalline and amorphous domains. b) Molecular structure of chitosan in the neutral and charged forms.

Following the design principles of tough hydrogels and applying them to NFC-based materials could offer viable routes for preparing strong and tough films that would retain attractive mechanical properties also in the wet state.²⁶ One such attractive pathway concerning NFC-based materials is physical crosslinking.^{27,28} Individual physical crosslinks are typically weaker than covalent ones, making them more likely to break upon stress, potentially serving as sacrificial bonds for dissipating energy during deformation.²⁹ Polysaccharides provide a widely examined class of polymers that bind with cellulose surfaces by multivalent supramolecular interactions and can function as physical crosslinkers for NFC.^{30,31,32,33}

Creating a transparent hybrid nanopaper of NFC and a polysaccharide requires that the components can be homogeneously mixed without major aggregation taking place.^{34,35} Mechanical stability of the structural material upon hydration demands sufficiently favorable interactions between NFC and the polysaccharide over those between water and NFC or the polysaccharide.

This combination of properties can be achieved with a stimulus-responsive polymer so that mixing can be carried out when the polymer is highly soluble in water and, during or after film preparation, can be insolubilized leading to mechanical stability under hydration. For this purpose chitosan, a pH-responsive polysaccharide that readily adsorbs on cellulose surfaces has been chosen.^{36,37,38}

Chitosan is a biocompatible polysaccharide produced by deacetylation from chitin, an abundant biopolymer mainly extracted from the exoskeleton of crustaceans.³⁹ Chitosan has drawn significant attention due to its attractive mechanical properties, sustainability, biocompatibility, and antimicrobial properties.^{40,41,42} The backbone of chitosan consists of *N-acetylglucosamine* and *D-glucosamine* units of which the latter contain primary amines which make the polymer pH-responsive. Chitosan is water-soluble when the amines are protonated, as shown in Figure 1, roughly below the pKa of the ammonium ion, between 6.0 and 6.5.^{43,44}

Here we report a simple route for preparing a transparent hybrid nanopaper with good mechanical stability under wet conditions. NFC is mixed with chitosan to form visually homogenous, viscous mixtures that are solvent cast and subsequently treated with a strong base to insolubilize chitosan. This leads to physical crosslinking of NFC and greatly enhances wet tensile properties without losing mechanical properties in the dry state.

Experimental

Materials. NFC was prepared from never dried birch pulp by disintegrating the pulp 20 times through a fluidizer (Microfluidics Corp., Newton, MA, USA) leaving a hydrogel with a consistency of approximately 2.11 wt.%. Chitosan from crab shells delivered as dry flakes of middle-viscosity ($M_w = 300\text{-}500$ kDa, degree of deacetylation = 80% determined by acid-base titration (see experimental section below) and high viscosity ($M_w = 500\text{-}700$ kDa, degree of deacetylation = 75%), 37% hydrochloric acid, sodium hydroxide and sodium sulfate were purchased from Sigma-Aldrich. Ammonium hydroxide (28-30%), acetic acid (>99.8%) and

tris(hydroxymethyl)aminomethane were acquired from Sigma-Aldrich. Ammonium formate and formic acid (98-100%) were acquired from Riedel-de Haën. Deionized water was obtained with a Milli-Q water system from Millipore.

Preparation of the buffer solutions. Buffer solutions were prepared by combining ammonium hydroxide with formic acid for buffers of pH 4.0 and 9.1 and tris(hydroxymethyl)aminomethane with acetic acid for buffers of pH 5.7 and 6.8. Ammonium formate was dissolved in deionized water at 1M. Ammonium hydroxide and formic acid were diluted to 1M with deionized water. 1M ammonium hydroxide or 1M formic acid was added to 1M ammonium formate until pH reached the desired value. Acetic acid was diluted to 1M and 2M stock solutions. Tris(hydroxymethyl)aminomethane was dissolved to a 2M stock solution with deionized water. 2M acetic acid and 2M tris(hydroxymethyl)aminomethane were combined in a 1:1 ratio to give 1M tris-acetate. For pH 5.7 tris-acetate buffer, 1M acetic acid was added to 1M tris-acetate until pH reached the desired value. 1M tris-acetate solution was used without further addition of acid or base for pH 6.8 tris-acetate buffer. Before use the 1M buffer solutions were diluted to 100 mM with deionized water. 100 mM buffer solutions with divalent anion for ionic crosslinking of chitosan were prepared by dissolving sodium sulfate in 100 mM buffer solutions to give 100 mM sodium sulfate. pH was measured with an electronic pH meter (6230N, JENCO).

Preparation of mixtures of NFC and chitosan and film casting. Chitosan was dissolved at 0.2 wt.% in 12 mM HCl, which had been diluted with deionized water. Dissolution time was 24 h at minimum. After no undissolved material was seen, the solution was filtered with a 5 μ m syringe filter (Arcodisc 25 mm Syringe Filter w/ 5 μ m Versapor Membrane, PALL). The NFC gel was diluted to 0.3 wt.% by adding deionized water followed vigorous stirring for 24 h. The diluted NFC suspension was then centrifuged at 5000 x g for 60 min to remove aggregates. The sediment was discarded and the supernatant was used for sample preparation. The mass concentration of NFC in the obtained supernatant was determined by weighing a given amount of the suspension and drying

it overnight in an oven at 50 °C followed by weighing. The final dry mass divided by the initial mass of the suspension to yield the mass percentage of the suspension. The values obtained were in the range of 0.17 % to 0.19 %. The used supernatant will be referred to as NFC in the rest of the text. Prior to mixing NFC was in deionized water and 0.2 wt.% chitosan in 12 mM HCl. Dissolved chitosan and NFC were combined by measuring a given amount of 0.2 wt.% chitosan solution in a glass bottle. NFC suspension was added until a desired dry mass ratio between chitosan and NFC was reached. The mixtures will hereafter be called NFC/chitosan [dry wt.% of NFC]/[dry wt.% of chitosan]. The acid concentration of the mixture was dependent on mixing ratio as the NFC suspension contained no acid. The total dry mass was approximately 120 mg and volumes were in the range of 50 – 80 mL. The mixture was homogenized with a high-shear homogenizer (Ultra Turrax T25 basic IKA Labortechnik) for 10 min at 11,000 rpm/min. Films were cast on polystyrene petri dishes (diameter 86 mm) followed by evaporation under ambient conditions in a fume hood on a horizontal surface. The dried films were cut in two halves of which one was immersed in 100 mM aqueous NaOH solution for 15 min followed by repeated rinsing with deionized water until pH of rinsing water was 7. This half will be hereafter referred to as “base-treated”. The other half was used as a reference sample and treated for 15 min with deionized water. This half will be hereafter referred to as “not base-treated” After draining excess water out the films were left to dry by evaporation in ambient conditions.

Tensile properties. The films were cut into strips 2.25 mm wide and more than 1.5 cm long by pressing with a custom made razor blade jig, where multiple flat blades are evenly spaced by flat metal spacers and tightened parallel to each other. For clamping, the strips were glued from both ends onto small pads of abrasive paper (ca. 3 mm x 4 mm) leaving a 10 mm long strip available for standard tensile tests. The widths of the strips were measured with an optical microscope (Leica MZ6 equipped with a Leica DFC420 camera). The thicknesses of the films were measured with a film thickness measurement set-up composed of a displacement sensor (LGF-0110L-B, Mitutoyo),

digital reader (EH-10P, Mitutoyo) and a measuring table with support for sensor (215-514 comparator stand, Mitutoyo). Wet samples were either first clamped to the tensile tester (Tensile/Compression Module 5kN with 100N load cell, Kammrath & Weiss GmbH) and soaked for 60 s with deionized water or soaked for 48 h in a buffer solution before measurement. Dry samples were equilibrated in a custom-made humidity chamber inside which also the tensile tester was located. Rate of elongation was 0.5 mm/min and gauge length was 10 mm. Optical video between crossed polarizers was recorded for most of the soaked samples. For this, the sample was illuminated from below through a polarizing film with the polarization direction at 45° angle with the sample elongation direction. Another polarizer was attached at the camera lens with 90° angle with the first polarizer.

Scanning electron microscopy (SEM). Dry films of supernatant and sediment of NFC after centrifugation were prepared by pipetting a small amount onto a silicon wafer attached to aluminum SEM stubs with carbon tape and sputtered with a thin gold film (Emitech K100X). Imaging was carried out with a Zeiss Sigma VP scanning electron microscope at 1-2 kV acceleration voltage.

Transmission electron microscopy (TEM). Micrographs were imaged in bright field mode with a Tecnai 12 Bio Twin instrument (FEI), operating at an accelerating voltage of 120 kV, and recorded with an Ultra-Scan 1000 CCD camera (Gatan). NFC/chitosan 80/20 w/w mixtures prepared from 0.18 wt.% NFC and 0.2 wt.% chitosan in 12 mM HCl were diluted to approximately 0.01 wt.% with deionized water. 3 µL of the diluted mixture was pipetted onto a copper TEM grid with a holey carbon film and allowed to stand for 60 s before draining the excess by blotting. Chitosan in the sample was subsequently stained with methyl iodide vapor for 10 min in a closed vial to enhance contrast.

Rheology. TA AR2000 stress controlled rheometer equipped with an aluminium double gap cylinder was used for rheological characterization. Oscillatory frequency sweeps were made in the linear viscoelastic range (2%) at 20 °C verified by strain sweeps. The flow properties were

measured from low to high shear rates. All measurements on NFC/chitosan mixtures were carried out on freshly prepared samples.

Determination of degree of deacetylation. The degree of acetylation of the chitosan samples was determined as previously reported.⁴⁵ Briefly, dried chitosan (0.2 g) was dissolved in 20 cm³ 100 mM HCl (aq.) and 25 cm³ water. After 30 min continuous stirring, water (25 cm³) was added and stirring for 30 min. Upon complete dissolution of chitosan, the solution was titrated with a 0.1 mM NaOH (aq.) solution and the degree of deacetylation (DA) according to Eq. 1:

$$DA, \% = 2.03 \frac{V_2 - V_1}{m + 0.0042(V_2 - V_1)} \quad (1)$$

where m is weight of sample in grams, V_1 and V_2 are the volumes of 0.1 mM NaOH (aq.) solution corresponding to the deflection points in cm³; 2.03 is a constant resulting from the molecular weight of chitin monomer unit and 0.0042 is a coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

Ultra violet-visible light (UV-vis) spectroscopy. Transmission spectra of thin films (approximately 12 μm thick) were recorded in UV-visible range with PerkinElmer Lambda 950 UV/Vis/NIR absorption spectrophotometer.

Wide-angle x-ray scattering (WAXS). WAXS measurements were performed on a Bruker AXS Nanostar-U Instrument equipped with a microfocus X-ray source (Incoatec ImSCu E025) operating at $\lambda = 1.54 \text{ \AA}$. A pinhole setup with 750 mm, 400 mm, and 1000 mm (from source to sample) was used and the sample-to-detector distance was 4.95 cm (calibrated with silver behenate). The scattering patterns were corrected for the beam stop prior to evaluations. Intensity distribution profiles in the azimuthal angle for 2θ values ranging from 18° to 24° (intensity maximum at 21°) wherein lies the (200) reflection, were used to calculate the Hermans orientation parameter (S) according to the Eqs. 2 and 3:^{46,47}

$$S = \frac{3}{2} \langle \cos^2 \Phi \rangle - \frac{1}{2} \quad (2)$$

with

$$\langle \cos^2 \Phi \rangle = \frac{\sum_0^\pi I(\Phi) \sin \Phi \cos^2 \Phi}{\sum_0^\pi I(\Phi) \sin \Phi} \quad (3)$$

Samples from base-treated NFC/chitosan 80/20 w/w films were prepared for WAXS similarly as those for tensile characterization. After clamping and soaking with deionized water, the films were stretched to a predetermined strain and excess water was drained by carefully touching with the corner of a tissue. After this the load was waited to stabilize in order to prevent shortening from shrinking. Finally the samples were relaxed and allowed to fully dry. Used strains were 5, 10, 15 and 20 %. The unstrained samples were taken directly after base treatment without further processing. Strains higher than 20 % led to rupture of the films during drying in tension. Some shrinking during drying was observed in the form of slight curling of the film.

Results and discussion

A homogenous mixture of NFC and chitosan is possible when chitosan remains soluble and NFC does not flocculate or aggregate. Electrostatic stabilization of NFC is reduced at low pH as carboxylic acids within the surface-bound residual heteropolysacchrides lose charge and NFC flocculates due to the suppression of the electrostatic colloidal stabilization. This can be seen by adding a small amount of acid to a NFC suspension (see Figure S1). Chitosan is soluble only in an acidic solution and aggregates from solution if pH is increased above its pKa, which is between 6.0 and 6.5, to deionize the primary amine of the chitosan backbone.^{43,44} Therefore a homogenous mixture of these components can only be achieved within a given pH window. This is somewhat complicated by the buffering capability of both the amines in the chitosan and the carboxylic acids in NFC combined with the possibility of stabilization of NFC by adsorbed chitosan.

Figure 2. Schematic representation of the colloidal behavior upon mixing of NFC/chitosan in a) low pH where NFC flocculates due to the surface-bound heteropolysaccharides, b) optimal pH where both components remain stable and c) high pH where chitosan aggregates.

Visually homogenous mixtures were achieved when using 0.2 wt.% chitosan dissolved in aqueous 12 mM HCl and 0.17 - 0.2 wt.% NFC in deionized water which resulted in a pH of 3.4. When 0.2 wt.% chitosan in 8 mM or 16 mM HCl were used, the mixture turned out inhomogenous (see Figure S2). The suggested behavior of the NFC/chitosan mixtures in solution of low, medium and high pH (pH 2.8, 3.4 and 4.5 respectively) is shown in Figure 2. Low pH (mixture pH 2.84, using 0.2 % chitosan in aq. 16 mM HCl) drives flocculation of NFC in solution. High pH (mixture pH 4.5, using 0.2 % chitosan in aq. 8 mM HCl) causes aggregation as chitosan crosslinks NFC prior to mixing. When pH is suitable (mixture pH 3.4, using 0.2 % chitosan in aq. 12 mM HCl), chitosan remains dissolved and flocculation of NFC is negligible. Chitosan adsorbed on NFC may provide further stabilization even at pH lower than what would make NFC alone to flocculate.

Figure 3. Method to prepare the NFC/chitosan mixtures and casting of hybrid nanopaper.

Visually homogenous and translucent NFC/chitosan aqueous mixtures were obtained using the preparation procedure shown in Figure 3. The interactions of NFC and chitosan upon mixing at 80/20 w/w ratio resulted in a slight increase in viscosity and turbidity and the mixture displayed a visible elastic response to agitation. This was confirmed by rheology by inspecting the storage (G') and loss (G'') moduli as function of angular frequency ω , see Figure 4a. For NFC/chitosan 80/20 w/w mixtures, $G' \sim \omega^0$ and $G' \gg G''$, indicating gelation. Also, pure NFC showed enhanced elasticity at low concentration, but the moduli were considerably lower. Pure chitosan was a very low viscosity fluid, with moduli that were in the lower limit of detection of the measuring unit, and are therefore not included in Figure 4a. The NFC/chitosan 80/20 w/w mixture showed much higher viscosity than the pure components at low shear rates indicating interactions between the components. The mixture and NFC were shear thinning, which has also earlier been observed for pure NFC dispersions.⁴⁸ Finally, we point out that the high viscosity values shown in Figure 4b suggested us to use low concentrations to prepare the mixtures, in order to obtain homogeneous mixtures even at very high NFC-to-chitosan ratios.⁴⁹

Figure 4. Oscillatory a) and flow b) behavior of NFC 0.18 wt.% supernatant in deionized water (black squares), 0.2 wt.% chitosan in 12 mM HCl (blue triangles), and mixture of NFC/chitosan 80/20 w/w (red circles). At these low concentrations, pure chitosan is an easily flowing liquid with low values of moduli, and not presented in the figure, as they cannot be reliably measured.

Mechanical properties. For the mechanical characterization, the films were cut in half and one part was immersed in 100 mM NaOH solution and the other in deionized water for 15 min followed by thorough rinsing with deionized water and drying in ambient conditions by evaporation. The prior sample is referred to as “base-treated” and the latter as “not base-treated”. Not base-treated NFC/chitosan 50/50 and 25/75 w/w films are excluded from the dataset due to excessive shrinkage and wrinkling, which prevented reliable mechanical testing. The dry films were cut and prepared for tensile characterization. After clamping the samples to the tensile tester they were fully soaked by pipetting deionized water on the samples and waiting 60 s before testing. This soaking time was

found to be sufficient as results were similar to base-treated samples soaked for 48 and 72 h (Figure S4).

Treatment with 100 mM NaOH solution has a clear effect on the wet tensile properties of all films at all mixing ratios, see Table S1. This drastic effect is illustrated for NFC/chitosan 80/20 w/w showing high wet tensile strength of nearly 90 MPa for the base-treated sample, which is a 15-fold increase in comparison to that of the not base-treated equivalent, see Figure 5b. Already the very low chitosan content of NFC/chitosan 95/5 w/w leads to a three-fold increase in the tensile strength under wet conditions and nearly doubling of the maximum strain. The tensile strengths and maximum strains in the wet state are significantly higher (e.g. roughly 9-fold higher tensile strength and 3-fold higher maximum strain for NFC/polymer 90/10 w/w composition) in the present work in comparison to those reached with covalent crosslinking of NFC by esterification with poly(acrylic acid).¹⁶ In Figure 5a the stress-strain curves in the wet state for different NFC/chitosan compositions after the base treatment are shown.

Figure 5. a) Effect of mixing ratio of NFC/chitosan for the wet tensile properties, following the base treatments. b) The wet tensile properties for base-treated and not base-treated NFC/chitosan 80/20 w/w.

Mechanism of crosslinking. In the dry state, the random NFC network is held together by mostly hydrogen bonds between the nanofibril surfaces. In the presence of water these physical bonds compete with water interactions leading to reduced direct interfibrillar hydrogen bonding.¹² It is known that water forms strong hydrogen bonds with polar solutes, such as carbohydrates.^{50,51} Overall, the combined effects manifest in a loss of macroscopic mechanical properties under wet conditions as the network is only poorly connected. The proposed mechanism of physical crosslinking of NFC by chitosan is qualitatively illustrated in Figure 6. It is suggested to occur through adsorption of chitosan onto NFC combined with subsequent insolubilization of chitosan by

the base treatment.³⁶ The primary amines in chitosan are deprotonated at high pH rendering chitosan uncharged and insoluble in water, thus desorption of chitosan from the NFC surface is significantly reduced. Hydration of a polyelectrolyte is reduced with reducing charge. This can be seen in osmotic potential of polyelectrolyte hydrogels.⁵² Upon deionization of chitosan, density of physical bonds between chitosan segments increase as the disrupting hydration layer is diminished and electrostatic repulsion is reduced. Bonding between NFC and chitosan is also believed to increase with reducing hydration of chitosan.

However, the exact mechanism of bonding between NFC and chitosan remains unclear. At least three interactions between cellulose and chitosan have been suggested: hydrogen bonding, ionic interactions and covalent imine linkage.⁵³ The presence of a specific, non-electrostatic interaction between cellulose and chitosan in solution has been shown.^{36,54} Entropic contribution from release of organized water at the hydration layer is likely to contribute to bonding.³³ The ionic interactions can be neglected because after immersion to 100 mM NaOH the chitosan backbone is uncharged.⁴³

Figure 6. Proposed mechanism of crosslinking of NFC network via chitosan in the wet state. a) Chitosan is charged at low pH leading to electrostatic repulsion and thicker hydration layers

between chitosan segments in comparison to b) neutral chitosan at high pH. Hydrogen bonding between chitosan segments is hindered by water as chitosan-chitosan interactions are in competition with water-chitosan interactions.

Effect of pH on the mechanical properties and ionic crosslinking. To investigate which interfaces are most affected by pH, a NFC/chitosan 80/20 w/w film was characterized under tensile loading after soaking in buffer solutions of different pH (9.1, 6.8, 5.7 or 4.0) for 48 h. The results are shown in Figure 7. The films become significantly stronger when pH exceeds the pKa of the ammonium ion in chitosan, which is between 6.0 and 6.5.^{43,44} At low pH (4.0 and 5.7) chitosan is highly charged and bonding between polymer chains is disrupted by electrostatic repulsion and an increased solvation shell around the chains. When a divalent sulfate anion is introduced into the buffer system, it is capable of ionically crosslinking the chitosan chains. This is seen in increases in the wet strength from below 5 to roughly 25 MPa and the wet strain from 10 to 18 %, see Figure 7b. If chitosan were to desorb upon dissolution at low pH from the NFC surface, the addition of an ionic crosslinker should not have such a drastic effect on the wet tensile properties. Thus, it can be claimed that at low pH only the interfaces between chitosan segments are weakened while chitosan remains strongly adsorbed onto NFC.

The above experiment suggests that the hybrid nanopaper becomes crosslinked, and thus strong in the wet state, upon bonding between chitosan segments. This can be induced by deionization of chitosan at high pH or ionic crosslinking of chitosan with a divalent anion. Multivalent hydrogen bonding and hydrophobic interactions are the main form of bonding between NFC and chitosan.^{30,31,32,55} Ionic interactions can be ruled out as their effect becomes negligible at the extreme pHs as either carboxylic acids in hemicellulose or amines in chitosan would be uncharged. A small amount of imine linkages between the aldehydes of the reducing ends of cellulose and primary amines of chitosan is possible, but constitute only a minor form of bonding between NFC and chitosan. If extensive covalent bonding via imine linkages were to occur, the system should be crosslinked

independent of the pH as single chitosan chains would be able to covalently bind to separate fibrils.⁵⁶

Figure 7. a) Influence of pH on wet tensile properties of NFC/chitosan 80/20 w/w composites. b) Ionic crosslinking of chitosan at low pH in the presence of divalent sulfate anions. Samples are soaked 48 h in 100 mM buffer before measurement.

Cyclic tensile characterization in the wet state was performed for NFC/chitosan 80/20 w/w samples to investigate whether the behavior is elastic or plastic, see Figure 8. The maximum strain per cycle was increased with small steps at low strain, which is highlighted in the inset in Figure 8. Already in the first strain cycle the behavior seems to be highly plastic as the stress during the second strain cycle begins to rise at a strain only slightly lower than the maximum strain of the previous cycle. After the first cycle the sample begins to demonstrate some elastic behavior also as

the roughly half of the strain is recovered. This behavior continues also to larger deformations until fracture. The material clearly demonstrates strain-hardening also in the wet state as the stress needed to apply to cause further plastic deformation increases.⁵⁷ Young's moduli are calculated for each elongation step during cyclic loading for three samples and shown in Figure S5.

In general dense, covalent crosslinking of NFC could lead to highly brittle behavior of the material, but this is avoided with physical crosslinking when using chitosan. This is highlighted by retaining the tensile stress-strain behavior of films of high strength also in the dry state (relative humidity 50%, temperature 20 °C) very similar to that of the neat NFC films shown in Figure 9. In fact, the film NFC/chitosan 80/20 w/w film is slightly softer and more extensible than the neat NFC film.

Figure 8. Cyclic tensile tests of NFC/chitosan 80/20 w/w composition in the wet state. The inset shows short cycles at low stress and strain.

Figure 9. Stress-strain curve of dry NFC/chitosan 100/0 and 80/20 w/w at 50% relative humidity.

Optical properties. Solvent casting of homogenous mixtures results in highly transparent films, of which few millimeter wide base-treated strips are shown in Figure 10 alongside their UV-vis transmission spectra. No visible difference in optical properties is seen between base-treated and not-base treated films. The films show high transparency between 60 % and 90 % over the visible spectrum, which is a significant improvement in comparison to previous work, where transparency is reduced to below 70% at already at 32% NFC and below 40% at 50% NFC.^{35,49} By visual inspection all films seem approximately equally transparent, but the pure NFC film has the highest transmission over the whole spectrum. The hybrid films have increasing transmission with chitosan loading. This may result from slight aggregation of NFC by chitosan during mixing. When the amount of chitosan in relation to NFC is low, an NFC fibril surface with adsorbed chitosan is more prone to bind with another NFC fibril without adsorbed chitosan. The transparency of the NFC/chitosan hybrid nanopaper is a result of the sub-wavelength dimensions of the formed structures.^{4,5} Centrifugation of the NFC suspension removes aggregates and poorly fibrillated material from the suspension as they are less stable than the residual nanofibrillar component as shown in Figure 3. This can also be seen from the scanning electron micrographs of the surfaces of films evaporated from the supernatant and sediment in Figure 11. The insets therein show the difference in transparency between the sediment and supernatant even after adjustment to equal

weight percent. In addition, the difference in transparency of the resulting films is shown in the inset. The aggregates and unfibrillated material lead to voids and more dense areas that are of the order of the wavelength of visible light and larger leading to diffraction. Homogenous mixtures and nanoscale dimensions of the components are shown in Figures S9, S10 and S11 which show TEM micrographs of NFC/chitosan 80/20 w/w with chitosan stained for 10 min in methyl iodide vapor.

Figure 10. Photograph of cut strips a) and UV-vis spectra b) of base-treated thin films (10-13 μm) for different NFC/chitosan mixing ratios. Arrows indicate the positions of the films of different compositions, which are difficult to resolve due to their transparency.

Figure 11. Scanning electron micrographs of films cast from a) supernatant and b) sediment of NFC after centrifugation for 1h at 5000 xg. Insets show corresponding suspensions both at 0.18 wt.% and films cast from the suspensions.

Orientation. Orientation can be induced in NFC films by drawing, as shown previously.^{58,59} Therein, special care was necessary to draw neat NFC films in the wet state due to their mechanical weakness. In our case, the drawing has been considerably easier as the films are significantly stronger in the wet state than neat NFC films. Due to the transparency of the films, it was possible to follow the development of the orientation *in-situ* between crossed polarizers during the tensile testing using video. Figure 12 shows video frames at different intervals showing the development of birefringence, which is proportional to Hermans orientation parameter $f = \frac{\Delta n}{\Delta n_0}$, where Δn is the birefringence of the material and Δn_0 is the maximum birefringence.⁴⁷ The colors seen between

crossed polarizers change smoothly upon stretching (Figure 12). We interpret them using Michel-Lévy (Figure S7) and Raith-Sørensen birefringence charts (Figure S8).⁶⁰ For example, the birefringence of the 12.1 μm thick film depicted in Figure 12 reached a value of $\Delta n = 0.034 - 0.051$ in the violet edge at 30% elongation. The birefringence is reduced upon rupture of the film and subsequent relaxation, which can be seen also in the last frame of Figure 12. After rupture Δn drops to roughly 0.015 – 0.030. These values could be compared with the commonly accepted value $\Delta n_0 = 0.074$ for a perfectly aligned crystal of native cellulose.⁶¹ The above consideration would render the Hermans orientation parameter to lie between 0.459 – 0.689 before and 0.203 – 0.405 after rupture. This magnitude of drop in birefringence by roughly a factor of two corresponds well with the elastic behavior seen in cyclic tensile tests, where elastic strain is roughly half of the total strain per cycle.

Figure 12. Optical images of wet NFC/chitosan 80/20 w/w under tension between crossed polarizers with illumination from below. Polarizer axes are at 45° to the sample elongation direction.

Orientation of the base-treated NFC/chitosan 80/20 w/w films was further examined using WAXS experiments. Samples were stretched up to 0, 5, 10, 15 and 20% strains in the wet state and subsequently dried. Hermans orientation parameters were calculated from intensity distribution profiles in the azimuthal angle along (200) reflection of the crystalline cellulose I_{β} .⁴⁶ Two exemplary diffractograms and their intensity distribution profiles in the azimuthal angle of samples with draw ratios 1.0 and 1.2 are shown in Figure 13a and b. The calculated orientation parameters of films versus their draw ratios are shown in Figure 13c. Measured orientation is negligible below draw ratio 1.1 and increases at higher draw ratios. The values are small compared to the literature as for comparable cold drawn NFC films a Hermans orientation factor of 0.36 was reported with a draw ratio of 1.2.⁵⁸ This is three-fold compared to the values obtained with a draw ratio of 1.2 in this work. The reason for this difference is not clear, but it is possible that the chitosan chains crosslinking NFC fibrils are able to function as entropic springs retracting the structure back towards its original conformation.

Figure 13. WAXS diffractograms of wet-stretched and dried base-treated NFC/chitosan 80/20 w/w films with draw ratios a) 1.0 and b) 1.2, corresponding to the intensity distribution profiles in the azimuthal angle (below diffractograms) and c) orientation parameter versus draw ratio of films. The WAXS diffraction is highlighted with an arrow along which the intensity distribution profiles in the azimuthal angle are taken. Note that in the intensity distribution profiles the baselines are not adjusted to zero and both profiles are on the same scale enabling easier evaluation of the respective degree of orientation.

Conclusions

Transparent hybrid nanopapers of NFC and chitosan were prepared and physically crosslinked to significantly improve the wet tensile properties of the films by reducing the water-solubility of one film component, chitosan, inside the films. Transparency (~ 80 % throughout the visible spectrum) resulted from homogenous mixtures, nanoscale dimensions of NFC fibrils and suppressed

aggregation in the films leading to reduced diffraction of visible light. Already small increases in chitosan content from 0% to 5% caused dramatic improvements in wet tensile properties when the film was treated with 100 mM NaOH solution. The highest wet tensile strength reached 100 MPa for the base-treated NFC/chitosan 80/20 w/w composition. The maximum strains of all base-treated compositions with chitosan reached at least 18 % and many strained up to 25 % without breaking. Tensile moduli grew with plastic strain from 4 and 14 GPa at low (0.5 %) and large (16 %) strains. Increasing chitosan content to 75 % increased the maximum strain further, but decreased tensile strength. The effect of pH on wet tensile properties was investigated and a clear increase in wet strength occurred for an increase in pH from 5.7 to 6.8, i.e., from below to above pKa of the chitosan ammonium ion. The wet strength of the base-treated NFC/chitosan was interpreted to result from a physical crosslinking mechanism where chitosan is tightly adsorbed onto cellulose surfaces via multivalent physical interactions. The physical crosslinking did not reduce the remarkable toughness of hybrid nanopapers and a strength of 200 MPa (with maximum strain of 8%) was measured at 50 % air relative humidity for base-treated NFC/chitosan 80/20 w/w film. The presented approach allows a simple concept for the NFC-based transparent films to reduce the humidity sensitivity of the mechanical properties for advanced applications.

Supporting Information Available

Supplementary information contains material about behavior of NFC upon exposure to dilute HCl, quality of produced mixture of NFC/chitosan 80/20 w/w prepared using chitosan with different concentrations of HCl, wet tensile stress-strain curves of base-treated NFC/chitosan 90/10 w/w soaked for varied period of time in deionized water, wet tensile stress-strain curves of base-treated NFC/chitosan mixtures with different molecular weights of chitosan, elastic moduli recorded from cyclic tensile tests for base-treated wet NFC/chitosan 80/20 w/w films, Michel-Lévy and

Raith- Sørensen birefringence charts and TEM micrographs of NFC/chitosan 80/20 w/w. This material is available at free of charge via the Internet at <http://pubs.acs.org/>.

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