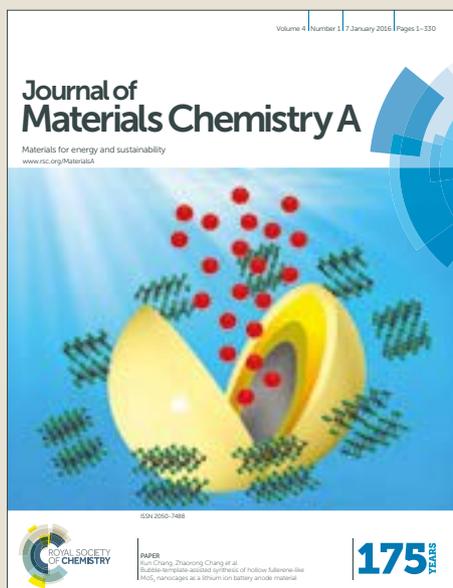


# Journal of Materials Chemistry A

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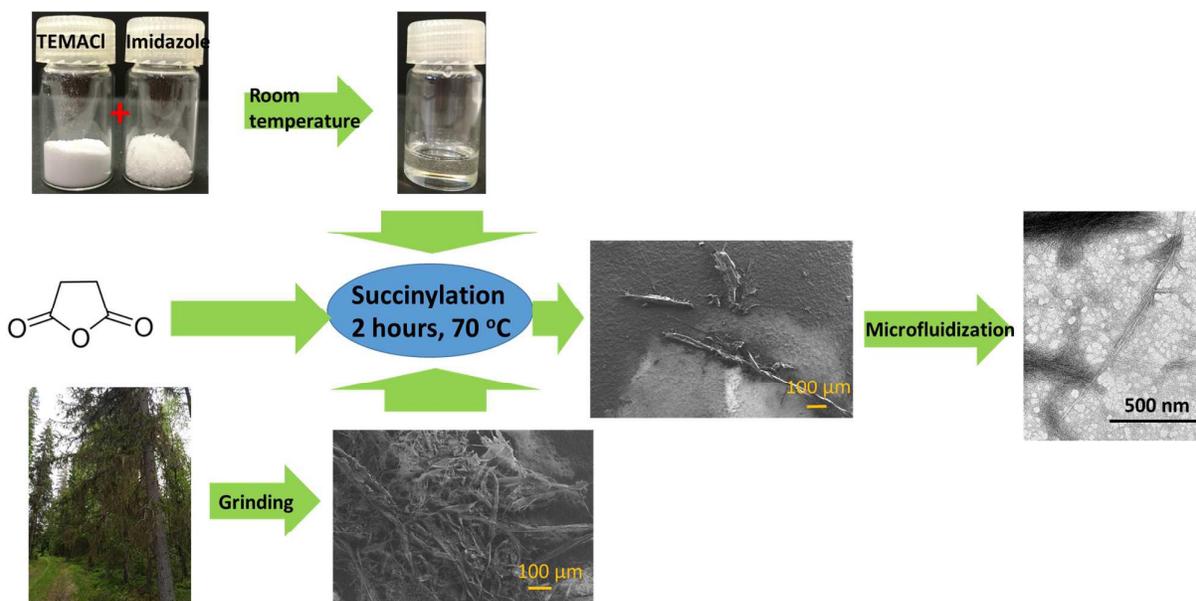


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Easily produced deep eutectic solvent was used as efficient reaction media for chemical modification of lignin-rich groundwood pulp to obtain highly charged anionic wood nanofibers.



## Journal Name

## ARTICLE

## Anionic wood nanofibers produced from unbleached mechanical pulp by highly efficient chemical modification

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Received 00th January 20xx,  
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chemical modification of lignocellulosic materials, especially as a pre-treatment in nanocellulose production, has mainly been conducted with lignin-free bleached cellulose pulps. However, non-bleached pulp exhibits several advantages over bleached pulp, namely excluding the use of hazardous bleaching chemicals, higher yield, and lower cost. In this study, the chemical modification of lignocellulose (groundwood pulp, GWP) with a high lignin content (27.4 wt%) was investigated using a deep eutectic solvent (DES) as a reaction medium. A low-melting DES was easily obtained within one hour by mixing triethylmethylammonium chloride (TEMACl) and imidazole at room temperature. Carboxylated GWP was obtained by adding succinic anhydride to the DES. In mild reaction conditions (2 h at 70 °C), carboxylic acid contents of 1.88–3.34 mmol/g were obtained depending on the anhydride dosage used (5–20 mmol/1 g of pulp) with excellent yield (over 90%). The GWP was more reactive in the pre-treatment step, measuring carboxylic acid contents higher than those of bleached cellulose pulps treated in identical reaction conditions (containing less than 0.5 wt% lignin). After deprotonation of the carboxylic acid groups, highly anionic wood nanofibers (AWNFs) were produced using a microfluidizer. Vacuum filtration was applied in the preparation of self-standing films, which had good mechanical properties and were transparent. The fabricated AWNFs have many potential uses—for instance, in sustainable water purification because of their adjustable surface charge.

### Introduction

Nanosized cellulosic materials are a fundamental part of the new biorefinery concept, which promotes the use of biobased materials in applications where oil-based or inorganic materials are currently used.<sup>1</sup> Nanocellulose, including long cellulose nanofibers (CNFs) and short, rod-like cellulose nanocrystals (CNCs), possesses advanced properties, including a light weight, large surface area, and high strength and modulus, which often surpass those of their synthetic counterparts. In addition, lignocellulose is the most abundant renewable biopolymer (cellulose and lignin being the first and second most abundant organic polymers on Earth, respectively) that can be obtained from non-food sources, and even from waste materials, thus providing a basis for sustainable material fabrication.

Currently, cellulose-based nanomaterials are mostly produced from low-lignin bleached chemical pulps. The use of non-bleached, mechanical pulp could provide many advantages, including a lower price (around 50% lower), a higher production yield (85–95%), and a reduced environmental burden (the use of environmentally harmful bleaching chemicals is eliminated) compared to bleached cellulose

pulps.<sup>2</sup> However, preliminary studies have indicated that the fabrication of nanocellulose from lignin-containing pulps is a cumbersome process,<sup>3</sup> and delignification has been required.<sup>4</sup> Recently, heat-intensified disc nanogrinding of mechanical pulp was introduced to produce wood nanofibers (WNFs).<sup>5</sup> In addition, acid hydrolysis of lignin-containing pulp has been shown to be an efficient way to produce both CNCs and CNFs.<sup>6</sup> However, information is still scarce about the production of chemically functionalized WNFs to obtain nanosized lignocellulose materials with an adjustable surface charge. Deep eutectic solvents (DESs) are a relatively new class of chemicals used as solvents, catalysts, and reagents.<sup>7,8</sup> DESs are generally produced by mixing two or more chemicals together at elevated temperatures. They are easily obtained from widely available components that exhibit low toxicity, do not need further purification, and in the best cases are biodegradable. DESs have been used in nanoparticle fabrication<sup>9–11</sup> and recently in nanocellulose fabrication.<sup>12–15</sup> In this study, a DES based on imidazole and triethylmethylammonium chloride (TEMACl) was used as a reaction medium for the chemical modification of non-bleached mechanical pulp (groundwood pulp, GWP). Succinic anhydride was chosen as a reagent, as it allows the production of carboxylated wood material through the formation of a half-ester of succinic acid. The reaction efficiency of the GWP was compared to that of bleached cellulose pulps. The carboxymethylated GWP was further mechanically disintegrated to obtain anionic wood nanofibers (AWNFs). The

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Electronic Supplementary Information (ESI) available: [TEM images of AWNFs with diameter and length measurements]. See DOI: 10.1039/x0xx00000x

mechanical properties of the AWNFs were then analyzed by measuring their tensile strengths from fabricated self-standing films.

## Materials and methods

Unbleached spruce GWP was obtained in never-dried form, whereas the reference pulps (softwood dissolving cellulose pulp and hardwood bleached birch pulp)<sup>16</sup> were obtained as dry sheets. The materials were first disintegrated in water, filtered, washed with ethanol, and dried at 60 °C for 24 h.

Imidazole, TEMACI, and succinic anhydride were purchased from TCI (Germany). Ethanol, NaOH (1 M), and HCl (0.1 M) were acquired from VWR (Finland) and phosphotungstic acid from Sigma Aldrich (Germany). All chemicals were used as received, without further purification. Durapore polyvinylidene fluoride membranes (pore size 0.65 μm) for the fabrication of self-standing films were acquired from Millipore (France). Deionized water was used throughout the study.

### Synthesis of DES

The DES was produced by combining TEMACI and imidazole into a container at a molar ratio of 3:7. After thorough mixing of the components by shaking in a closed vessel, the DES was allowed to stand at room temperature until a turbid solution was formed (about 10 min). The suspension was then mixed with a magnetic stirrer until a clear solution was obtained.

### Succinylation of PWG in DES

For the succinylation of the GWP, 26.35 g of TEMACI and 23.65 g of imidazole were weighted into a beaker and mixed in an oil bath at 70 °C for about 10 min until a clear, low-viscosity solution was obtained. The GWP (2 g) was added and mixed for 1 min, followed by the addition of succinic anhydride (0.5, 1, or 2 g). The reaction was allowed to proceed for 2 h under constant mixing. The beaker was then removed from the oil bath, and ethanol (50 ml) was added during mixing. The resulting mixture was filtered and washed with ethanol (200 ml), 0.1 M HCl (100 ml), and deionized water (100 ml). A similar treatment was also performed without the addition of succinic anhydride, and reference succinylation was performed on the birch and on the dissolving pulp.

### Determination of acidic groups

The carboxyl content of the ethanol-washed raw pulps and succinylated samples was analyzed by conductometric titration using a protocol described by Rattaz et al. and Katz et al.<sup>17,18</sup>

### Disintegration of succinylated cellulose to nanofibrils

The succinylated GWP was diluted to a consistency of 1 wt% in deionized water, after which the desired amount of NaOH was added (at a 1:1 molar ratio between NaOH and the carboxylic acid content of the GWP), and the GWP was further diluted to a 0.5 wt% consistency. An Ultra Turrax treatment (10 000 rpm) was then applied for 1 min. A microfluidizer (Microfluidics M-110EH-30, USA) was used to nanofibrillate the chemically modified pulp. All the samples were passed twice through 400

and 200 μm chambers at a pressure of 1300 bar and four times through 200 and 87 μm chambers at a pressure of 1500 bar.

### Preparation of self-standing films from nanofibers

The AWNFs were used to prepare self-standing films for characterization of the mechanical properties of the nanofibers. First, 0.33 g abs. of nanofibers were diluted to 0.3 wt% (corresponding films with a grammage of 80 g/m<sup>2</sup>) and were further mixed with a magnetic stirrer to ensure the formation of evenly dispersed suspensions. Vacuum filtration with a glass filter funnel (7.2 cm in diameter) covered with a polyvinylidene fluoride (PVDF) membrane (pore size 0.65 μm) was applied for the fabrication of gel "cake." The gel was then covered with another PVDF membrane and dried between two paper sheets for 9 min at 93 °C under a vacuum at about 70 mbar of pressure.<sup>19</sup>

### Attenuated total reflection infrared spectroscopy

The chemical characterization of the pristine and succinylated GWP was performed using an ATR-IR. The spectra were collected from dried and pressed samples using a Bruker Tensor II FT-IR Spectrometer with a Hyperion 3000 FT-IR Microscope (USA). Spectra were obtained in the 600–4000 cm<sup>-1</sup> range, and 40 scans were taken at a resolution of 4 cm<sup>-1</sup> for each sample.

### Transmission electron microscopy

The morphological features of the fabricated AWNFs were analyzed with a JEOL JEM-2200FS transmission electron microscope (Japan). Each sample was prepared by dilution with ultrapure water. A carbon-coated copper grid was then prepared by coating with polylysine. A small droplet of a 0.1% solution of polylysine was placed on top of the grid and allowed to stand for 3 min. The excess polylysine was removed from the grid by touching the droplet with one corner of a filter paper. A small droplet of the diluted AWNF sample was then placed on top of the grid and the excess of the sample removed by the method described above. Samples were negatively stained with phosphotungstic acid (2% w/v at pH 7.3) by placing a droplet on top of each specimen. The excess phosphotungstic acid was removed as described above. The grids were dried at room temperature and analyzed at 200 kV under standard conditions. The dimensions of the AWNFs were measured using the TVIPS EM measuring program (beta 0.85).

### X-ray diffraction

The crystalline structures of the original pulps and CNCs were investigated using wide-angle X-ray diffraction. Measurements were conducted on a Rigaku SmartLab 9kW rotating anode diffractometer (Japan) using a Co K $\alpha$  radiation (40 kV, 135 mA) ( $\lambda = 0.17903$  nm). Samples were prepared by pressing tablets of freeze-dried celluloses to a thickness of 1 mm. Scans were taken over a  $2\theta$  (Bragg angle) range from 5°–50° at a scanning speed of 10°/s, using a step of 0.5°. The degrees of crystallinity in terms of the CrI were calculated from the peak intensity of the main crystalline plane (200) diffraction ( $I_{200}$ ) at 26.2° and

from the peak intensity at 22.0° associated with the amorphous fraction of cellulose ( $I_{am}$ ), according to Eq. (1)<sup>20</sup>:

$$CrI = \left( \frac{I_{200} - I_{am}}{I_{200}} \right) \cdot 100\% \quad (1)$$

It should be noted that the Segal method used for CrI calculations is not suitable for the comparison of different types of samples but rather quantifies changes within a single sample set.<sup>21</sup>

The average size of crystallite ( $L$ ) was calculated from the Scherrer equation (2)<sup>22,23</sup>:

$$L = \frac{K \times \lambda}{\beta \times \cos \theta} \quad (2)$$

where  $K$  is a constant value 0.74,  $\lambda$  is the X-ray wavelength (0.17903 nm),  $\beta$  is the half-height width of the diffraction band (200); and  $\theta$  is the Bragg angle corresponding to the (200) plane.

### Mechanical properties of self-standing films

The tensile tests were performed with a universal material-testing machine (Zwick D0724587, Switzerland) equipped with a 100 N load cell. The films were cut into strips 5 mm wide, and their thicknesses were measured using a precision thickness gauge (Hanatek FT3, UK). Three different locations within the gauge length were measured to calculate the average thickness of each film. For the tensile tests, a 40 mm gauge length was set under a strain rate of 4 mm/min, and five strips in total were measured for each sample. The tests were conducted in a relative humidity of 50% at a temperature of 23 °C using a prestrain of ~0.1 N. Prior to testing, each specimen was conditioned for at least one day in this environment.

## Results and discussion

The DES used in this study was produced by mixing together TEMACI and imidazole at a molar ratio of 3:7. Almost immediately after the two components were mixed, wetting of the system was observed. In addition, the reaction vessel cooled to some extent, indicating a rapid endothermic reaction between the two components. Clear, slightly viscous DES was obtained within one hour under mixing in a closed vessel at room temperature (Figure 1). The formation of a DES at room temperature is desirable, as the heating often required for DES fabrication consumes a significant amount of energy and can result in heat-induced degradation of the DES chemicals.<sup>24</sup>

This DES, based on TEMACI and imidazole, remained liquid, albeit highly viscous, even at -24 °C, while the DES based on imidazole and choline chloride has been reported to have a melting point of 56 °C with the same molar ratio as was used here.<sup>25</sup> Because of its easy preparation from commercially available chemicals and its improved liquid range (according to the supplier, the melting points of imidazole and TEMACI are 88–91 °C and 282–284 °C respectively), this DES was chosen as a potential reaction medium for the succinylation of GWP. Both chemicals are also considered safe, and imidazole has previously been investigated as a green solvent for the

dissolution of starch<sup>26</sup> and a recyclable solvent in biomass fractionation.<sup>27</sup> However, as the formation of the DES can alter the toxicity profile of its components,<sup>28</sup> it is recommended that the DES used here be considered and handled as a toxic chemical pending further investigation.



Figure 1. TEMACI, imidazole, and DES a) directly after mixing by shaking, b) after standing 10 min, and c) after mixing with a magnetic stirrer for about 45 min at room temperature.

A high carboxylic acid content was achieved after the succinylation of GWP. Even with low succinic anhydride loading (5 mmol/g of pulp), a 1.88 mmol/g (Sample 1) carboxylic acid content was achieved (Figure 2). Through increasing the amount of succinic anhydride to 10 and 20 mmol/g of pulp, the carboxylic acid content was increased to 2.45 (Sample 2) and 3.34 (Sample 3) mmol/g, respectively. Significant growth in the mass of the pulp was observed after the reactions and yields of the succinylated GWP reached 93%, 92%, and 95% for Samples 1, 2, and 3 respectively. These high yields indicate that only a minimal amount of material was dissolved during the reaction and washing. When GWP was pre-treated by DES without the addition of succinic anhydride, the yield was 89%.

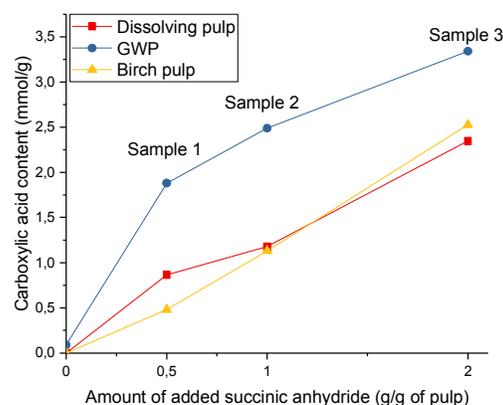


Figure 2. Carboxylic acid contents of succinylated GWP, dissolving pulp, and birch pulp in relation to added succinic anhydride.

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Previous studies on the chemical modification of lignin containing pulp (thermomechanical pulp, TMP) using TEMPO-NaBr oxidation system together with 7.5 mmol/g of NaClO resulted in formation of lignocellulosic pulp with a carboxylic acid content of 1.2 mmol/g.<sup>29</sup> The method also led to delignification of the pulp and when using significantly high content of NaClO (13 – 26 mmol/g) almost complete delignification occurred (the carboxylic acid content of the oxidized pulps were between 1 and 1.5 mmol/g).<sup>30</sup> Therefore, the current method is more suitable for producing carboxylated lignocellulose pulp with high carboxylic acid content, yield, and minimal lignin loss.

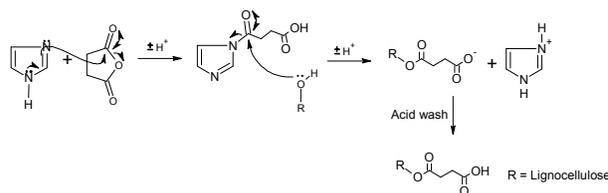
The reaction efficiency, designating the amount of reacted succinic anhydride, was 56%, 49%, and 50% for Samples 1, 2, and 3 respectively. Therefore, around half of the succinic anhydride reacted, and the rest either remained unreacted or was consumed in side reactions, including the ring-opening reaction with water to form succinic acid. Even though no water was added to the reaction system, all of the chemicals, including the GWP, are hygroscopic, resulting in the presence of small quantities of water. However, the reaction efficiency can overall be considered acceptable, especially when high yields are produced.

The reference bleached pulps had lower reactivity in succinylation (Figure 2). With the lowest succinic anhydride loading, the carboxylic acid content of succinylated dissolving pulp was about two times lower than that of the GWP. In the case of the birch pulp, an even lower carboxylic acid content (0.48 mmol/g) was attained. When the amount of succinic anhydride was increased, both bleached pulps exhibited similar carboxylic acid content, i.e., about 1 mmol/g lower than that of the GWP. Based on the succinylation of the reference pulp, it can be concluded that the presence of lignin strongly impacts the reaction's efficiency. Compared to cellulose, lignin is an amorphous polymer, which could allow it to interact better with succinic anhydride than cellulose does. When the reaction efficiencies of dissolving pulp and birch pulp (with hemicellulose contents of 3.5% and 24.7% respectively) are compared, it appears that the amount of hemicellulose does not contribute significantly to succinylation efficiency, especially with high anhydride loading.

With lignin-free bleached pulp, a carboxylic acid content of 3.8 mmol/g has been reported for succinylated fibers.<sup>31</sup> In the current study, lower reactivity (2.5 mmol/g) was achieved with birch pulp (which has a similar chemical composition). However, a higher temperature (90 °C), longer reaction time (5 h), and greater loading of succinic anhydride (21.5 mmol/g of pulp) were applied in the previous study.<sup>31</sup> Interestingly, in the current study, the carboxylic acid content of the dissolving pulp was higher than with a previously reported DES based on urea and lithium chloride, even though a succinic anhydride loading over 12 times lower was used.<sup>15</sup> The higher reaction efficiency might be due to the catalytic effect of imidazole.<sup>32</sup>

It has been proposed that the reaction between imidazole and anhydrides forms *N*-acylimidazole, which functions as an intermediate. The formed intermediate can then react with the hydroxyl groups in lignocellulose to form a half-ester of

succinic acid (Scheme 1). When imidazole is used as part of the solvent, the presence of a high excess of imidazole can significantly enhance the reaction's efficiency. On a side note, *N*-acetylimidazole (*N*-acylimidazole with an acetyl group) is known to selectively react with the primary hydroxyl groups of sugars,<sup>33</sup> implying that succinylation in a DES might be useful for the regioselective modification of hydroxyl groups. However, because of the complex reaction system involved, more detailed study is required to confirm this.



Scheme 1. Possible reaction mechanism of the imidazole-catalyzed succinylation of GWP in a DES via formation of *N*-succinylimidazole intermediate.

An ATR-IR spectrum of the GWP showed typical O-H and C-H stretching peaks at wavenumbers 3355 and 2892 cm<sup>-1</sup> (Figure 3). Other characteristic peaks of lignin- and hemicellulose-rich lignocellulose pulp were observed at 1735 cm<sup>-1</sup> (C=O stretching of the ester from the acetyl group of hemicellulose and ester linkage of the carboxylic groups in the ferulic and *p*-coumeric acids of lignin/hemicellulose),<sup>34</sup> 1605 and 1508 cm<sup>-1</sup> (C=C stretching of the aromatic ring in lignin),<sup>35</sup> and 1264 cm<sup>-1</sup> (the C-O aryl group of lignin). A highly intensive peak at wavenumber 1728 cm<sup>-1</sup> rose after succinylation. The peak was related to a superimposed band of the C=O group of the ester and the protonated carboxylic acid. This is typical of the half-esters of dicarboxylic acids of natural polymers such as cellulose.<sup>36</sup> In addition, an intensive C-O band of antisymmetric stretching in ester groups was seen at 1157 cm<sup>-1</sup>.<sup>37</sup> A sodium salt of the succinylated GWP exhibited a new peak at 1576 cm<sup>-1</sup>, corresponding to the C=O band of the deprotonated form of the carboxylic acid group. The characteristic peak for lignin (at 1605 and 1508 cm<sup>-1</sup>) occurred in the spectra for both the protonated and deprotonated forms of the succinylated GWP, confirming the presence of lignin after the chemical modification. Based on the intensity of the peak at 1508 cm<sup>-1</sup> of the normalized spectra, an average of 71% of the lignin was still present in the succinylated GWP. There was no significant difference in the peak intensity between the protonated and deprotonated forms of succinylated GWP, indicating that anionization does not alter the dissolution rate of lignin. This was also supported by the fact that the yield did not decrease when succinylated GWP was anionized at pH 8, followed by filtration and washing with water.

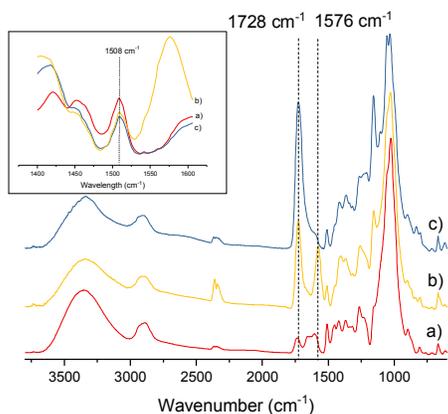


Figure 3. Normalized ATR-IR spectra (off-scale) of a) unmodified GWP, b) deprotonated GWP, and c) protonated succinylated GWP (carboxylic acid content of 1.88 mmol/g). Insert: On-scale spectra of the region showing the intensity of C=C stretching of the aromatic rings of lignin.

The chemical modification of the GWP did not alter the crystalline structure of the cellulose, but the XRD diffraction patterns (Figure 4) indicated a decrease in crystallinity. After succinylation, the crystallinity of the GWP reduced from 47% to 37–38%. A similar decrease in crystallinity, although lower, was observed with the dissolving pulp and birch pulp, as their CrI decreased from their original values of 66% and 64% respectively to 59% and 60% respectively. The crystallinity of GWP is based on the presence of semi-crystalline cellulose (lignin and hemicellulose are both amorphous polymers.) Therefore, the decline in crystallinity might indicate that a small amount of cellulose was dissolved during the reaction or in the following washing step. It is also possible that succinylation takes place in the crystalline parts of cellulose, disturbing the packaging of cellulose molecules and thus lowering the cellulose's crystallinity. However, it should be noted that because of the high substitution degree, a significant amount of the specimen consisted of succinyl moiety, which might appear as amorphous or only semi-crystalline. This would then lead to a decrease in the overall crystallinity of the sample even though the crystallinity of the cellulose would remain intact. When GWP was treated with a DES without succinic anhydride, its crystallinity increased to 58%. This is consistent with the yield lost during the treatment with pure DES, which indicates the dissolution of amorphous lignin/hemicellulose. In addition, the average crystallite size was not altered significantly during the DES treatment without succinic anhydride (original GWP and DES treated pulp had average crystallite sizes of  $4.37 \pm 0.04$  nm and  $4.22 \pm 0.02$ ). However, the average crystallite size decreased to  $2.83 \pm 0.12$  nm. Similar changes in the crystallite sizes have been previously reported for chemically modified bacterial cellulose.<sup>38</sup> Dimensional changes might be due to the reaction occurring on the surface of crystallites.

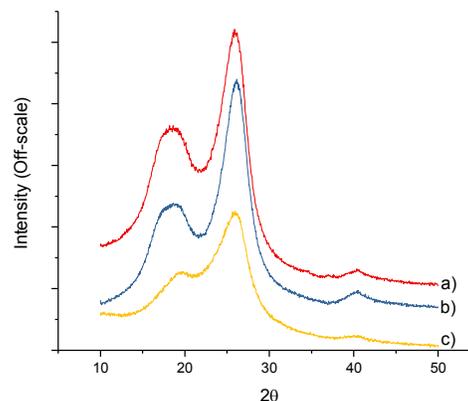


Figure 4. XRD diffraction patterns of a) the original GWP, b) GWP treated with DES without succinic anhydride, and c) GWP succinylated using a succinic anhydride loading of 20 mmol/g of pulp.

Succinylation has previously been used as a pre-treatment to improve the nanofibrillation of bleached cellulose via anionization of its carboxylic acid groups at neutral and alkaline pH.<sup>15,31</sup> Succinylated GWPs were nanofibrillated with a microfluidizer to obtain AWNF1, AWNF2, and AWF3 with carboxylic acid contents of 1.88, 2.45, and 3.34 mmol/g, respectively. After the nanofibrillation, low viscose, turbid and slightly yellowish solutions were obtained (Figure 5). All of the samples exhibited tendency for sedimentation, and after 48 hours AWNF2 and AWF3 formed visible layer on the bottom of container and the solutions became more transparent. AWNF1 started to deposit sediment more slowly, and after 48 h, only a small amount of clear water was visible at the top of the container.

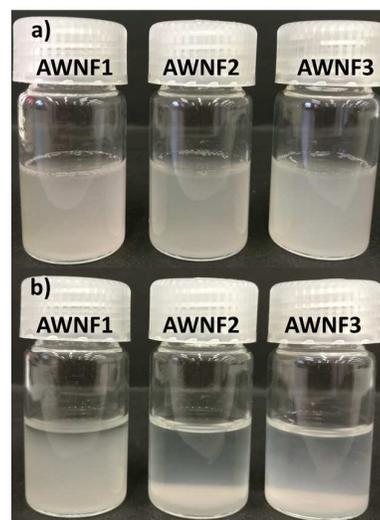


Figure 5. AWNF suspensions (1%) a) directly after preparation and b) after standing for 48 h at room temperature.

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Based on analysis of TEM images of the samples, the AWNFs had a heterogeneous size distribution (examples of TEM images of AWNF2 are presented in Figure 6; more TEM images of all the AWNF samples can be found in the supplementary information). In AWNF1, individual nanofibers with diameters around 5 nm were clearly seen, and larger nanofiber bundles with diameters around 20–500 nm were also present. In addition to nanosized fibers, some microfibers with diameters close to 1  $\mu\text{m}$  remained after the microfluidization. As with the nanofibers obtained from bleached chemical pulp, long fibers (in micrometric scale) could be seen in AWNF1. On the other hand, AWNF2 and especially AWNF3 contained a higher number of short fiber bundles or aggregates along with long fibers similar to those observed in AWNF1.

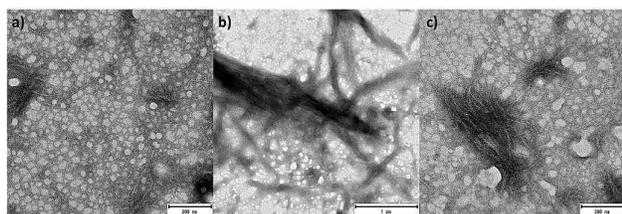


Figure 6. Examples of the TEM images of AWNF2: a) individual nanofibers with diameters of 5 nm, b) long fiber bundles with diameters from 20 nm to close to 1  $\mu\text{m}$ , and c) short fiber bundles.

Based on the TEM images, it was evident that the introduction of a high anionic surface charge alone was not enough to enable the liberation of evenly sized nanofibers, which have previously been obtained using bleached cellulose pulps. More uniform size distribution was recently reported after the mechanical disintegration of acid-hydrolyzed unbleached hardwood chemical pulp (with an initial lignin content of 17.2%).<sup>6</sup> The incorporation of initial grinding or partial chemical removal and the opening of the lignin structure could improve the efficiency of succinylation with a larger surface area and increase the liberation of individualized AWNFs. The appearance of AWNFs was, however, in line with the previous result obtained by heat-intensified disc nanogrinding of the same GWP.

The produced self-standing films exhibited excellent mechanical properties. AWNF1 and AWNF2 had tensile strengths over 100 MPa, whereas AWNF3 exhibited a slightly lower strength of 72 MPa (Table 1). As observed in the TEM images, AWNF3 contained a higher number of short but thick nanofiber bundles or aggregates. Short and thick fiber bundles have low aspect ratios and cannot entangle with other fibers, which might cause the lower tensile strength of AWNF3 compared to the other two samples. The presence of short fibers can, on the other hand, cause less absorption and reflection of light, which can be seen in the transparency, as films from AWNF2 and AWNF3 were more transparent than those from AWNF1 (Figure 7). AWNF2 had a strain around 70% higher and could therefore be suitable for applications requiring high tensile strength and moderate flexibility. Overall, films produced from succinylated GWP showed similar or even better (depending on the film fabrication method)

tensile properties compared to the previous films produced by heat-intensified disc nanogrinding of the same GWP.<sup>5</sup> The tensile strengths of the AWNFs were in the same range as, though slightly lower than, those of CNF films obtained from non-chemically modified, DES-treated bleached fibers.<sup>12</sup> The tensile strengths of nanopapers with lignin contents of  $\sim 14\%$  have previously been reported as  $116 \pm 7$ , which is similar to the properties of AWNF1.<sup>39</sup> The AWNF films had tensile strengths comparable to those of previously studied 2,2,6,6-Tetramethylpiperidin-1-yloxy (TEMPO)-oxidized CNF films produced from bleached fibers.<sup>40</sup> However, it should be noted that films produced from TEMPO-oxidized CNFs have also been reported to have significantly higher tensile strengths.<sup>41</sup> Also, CNF films based on sequential periodate oxidation and further chemical modifications have been shown to exhibit higher mechanical properties.<sup>40</sup>

Table 1. Mechanical properties and thicknesses of self-standing films produced from AWNFs.

Sample	Elastic modulus (GPa)	Maximum tensile strength (MPa)	Elongation at break (%)	Thickness ( $\mu\text{m}$ )
AWNF1	$6.2 \pm 0.26$	$113 \pm 3.7$	$3.3 \pm 0.5$	$64 \pm 2.8$
AWNF2	$5.6 \pm 0.26$	$107 \pm 3.4$	$5.6 \pm 0.5$	$57 \pm 1.9$
AWNF3	$4.1 \pm 0.31$	$72 \pm 6.2$	$5.7 \pm 0.5$	$53 \pm 2.2$

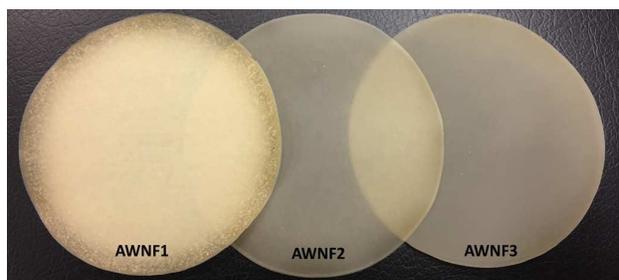


Figure 7. Self-standing films produced from AWNFs using vacuum filtration.

In addition to being used to produce self-standing films, surface-functionalized AWNFs could be suitable as flocculants in water purification<sup>42</sup> or the removal of toxic heavy metals<sup>43</sup> and organic dye molecules from water.<sup>44</sup> The presence of lignin can also provide some enhanced properties, such as  $\pi$ - $\pi$ <sup>45</sup> or cation- $\pi$ <sup>46</sup> interaction between lignin-containing nanocellulose and harmful chemicals (e.g., organic dyes), which could improve water purification efficiency. The use of non-bleached pulp instead of bleached cellulose fibers reduce the environmental burden of the production of wood-based nanomaterials. Especially the exclusion of the toxic and hazardous bleaching chemicals are advantageous properties of AWNF. In addition, non-bleached pulp can be considered more economical and sustainable, as it has low cost and higher yield compared to bleached pulp. Also the used DES exhibits several advantages over previously used reaction media, such as urea-LiCl DES, namely significantly

higher reactivity (observed with dissolving pulp), wide liquid range and formation at room temperature. Compared to traditional organic solvent TEMACI-imidazole DES also showed comparable properties as a reaction media for cellulose succinylation.

From the environmental point of view, succinylation can be regarded as a more sustainable way to produce anionized nanocelluloses than methods using halogen-based stoichiometric oxidants. In the industry, succinic acid, a precursor for succinic anhydride, can be produced in an environmentally friendly way via biomass fermentation.<sup>47</sup> Furthermore, even though succinic anhydride is produced by the dehydration of succinic acid using other anhydrides (e.g., acetic anhydride) at a larger scale, other, more sustainable routes are being studied, including a high-yield process using a heterogeneous Nb<sub>2</sub>O<sub>5</sub>·nH<sub>2</sub>O catalyst.<sup>48</sup> However, it should be noted that several other aspects, including the regeneration and purification of the DES after the reaction, should be investigated to fully achieve the sustainable chemical modification of lignocellulosic materials.

## Conclusions

An easily obtained DES based on imidazole and TEMACI was found to be an efficient reaction medium for the chemical modification of GWP with a high lignin content. The used DES was easily obtained with low energy consumption as the liquid state could be obtained at room temperature after short mixing time (less than one hour). The GWP exhibited a higher reactivity compared to bleached cellulose pulp, and a substantial number of carboxylic acid groups (up to 3.34 mmol/g) could be introduced at relatively mild reaction conditions (2 h at 70 °C) using succinic anhydride. The succinylated GWP was obtained with excellent yield (over 90%). After neutralization, the high anionic charge of the succinylated GWP enabled the production of nanosized lignocellulose material, and self-standing films with excellent mechanical properties could be produced from the AWFNs. This method provides a highly efficient and sustainable route to producing anionic nanomaterial with possible uses as a film material or in water purification. Especially the use of non-bleached pulp was advantageous over bleached cellulose fibers due to the lower environmental burden, higher yield, and lower production costs.

## Acknowledgements

We thank Kalle Hyypiö and Jani Österlund for their contributions at the experimental stage of the study. Dr. Juha Heiskanen is acknowledged for his help with the reaction mechanism, and Marcin Selent and Sami Saukko are acknowledged for their guidance with XRD and TEM analysis, respectively.

## Notes and references

‡ Footnotes relating to the main text should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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