

Combining steam explosion with 1-ethyl-3-methylimidazolium acetate treatment of wood yields lignin-coated cellulose nanocrystals of high aspect ratio

Hatem Abushammala · Ruben Goldsztayn ·
Alcides Leao · Marie-Pierre Laborie

Received: 6 January 2016 / Accepted: 9 March 2016 / Published online: 14 March 2016
© Springer Science+Business Media Dordrecht 2016

Abstract Lignin-coated cellulose nanocrystals (CNCs) were extracted from the tropical wood Angelim Vermelho in a yield of 47 % based on wood cellulose content. The procedure combined a mild steam explosion process with a treatment using 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]). The resulting CNCs showed exceptional aspect ratios of 83 ± 18 for wood-based CNCs and crystallinity index of 76 %. Residual lignin coating and cellulose acetylation were detected during the procedure and are proposed to minimize the known crystallite thickening. As a result, CNCs in dimensions close to their native state in wood were recovered. Furthermore, this novel extraction of CNCs directly from wood circumvents the tedious purification process necessary for CNC extraction from pulp.

Keywords Cellulose nanocrystal · Steam explosion · Ionic liquid · Wood

Introduction

We have reported earlier on the extraction of cellulose nanocrystals (CNCs) directly from wood using the ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) (Abushammala et al. 2015). [EMIM][OAc] ensured the multiple functions of delignifying wood and hydrolyzing wood amorphous holocellulose while at the same time it preserves cellulose native crystallinity. The procedure was remarkable in terms of process parameters and CNC attributes. It was (1) a direct approach from wood, (2) required only one reactant, (3) operated at mild conditions (temperature and time), with (4) no need for tedious purification steps such as dialysis. In terms of CNC yield and properties: The CNCs (1) were obtained in a relatively high yield (60 % of the wood cellulose) and (2) had a high aspect ratio (64 ± 28) with (3) no significant crystallite thickening (thickness = 2.6 ± 1.4 nm). In comparison, traditional CNC extraction methods require pure forms of cellulose as a starting material (such as pulp and microcrystalline cellulose) and extract CNCs in a yield of 10–40 % with an aspect ratio of 20–40 (Beck-Candanedo et al. 2005; Bondeson et al. 2006; Dong et al. 2012; Hamad and Hu 2010).

To explain the efficacy of this technology and the apparent recovery of nanocrystals in sizes very similar to native cellulose crystallites, we proposed that hydrolysis of lignin-carbohydrate complexes (LCCs)

H. Abushammala · R. Goldsztayn · M.-P. Laborie (✉)
Chair of Forest Biomaterials, Faculty of Environment and
Natural Resources, University of Freiburg, Werthmannstr.
6, 79085 Freiburg, Germany
e-mail: marie-pierre.laborie@biomat.uni-freiburg.de

H. Abushammala · R. Goldsztayn · M.-P. Laborie
Freiburg Materials Research Center, University of
Freiburg, Stefan-Meier-Str. 21, 79104 Freiburg, Germany

A. Leao
Department of Natural Resources, Sao Paulo State
University, Botucatu, Sao Paulo 18610-307, Brazil

(King et al. 2014), and acetylation of wood polymers favored a demixing or phase separation of wood polymers (Zweckmair et al. 2015), which together with the hydrolyzing power of the IL (Gazit and Katz 2012) resulted in CNCs separation (Abushammala et al. 2015). Also critical to the proposed mechanism, the acetylating power of [EMIM][OAc] allows derivatizing lignin phenolic hydroxyls thus deactivating its propensity for recondensations (Abushammala et al. 2015; Çetinkol et al. 2010; Ebner et al. 2008; Hauru et al. 2013; Karatzos et al. 2012; King et al. 2014; Köhler et al. 2007).

If this mechanistic hypothesis is true, then any pretreatments known to break LCCs should facilitate CNCs production with [EMIM][OAc]. Among the pretreatments capable of breaking LCCs, steam explosion is a well-established deconstruction process for wood (Mason 1928; Ruiz et al. 2008) known to enhance lignocellulose accessibility (Grous et al. 1986). In a typical steam explosion process, the lignocellulose is first soaked into a dilute aqueous alkaline (Kaushik et al. 2010) or acidic solution (Cherian et al. 2008), and then steamed under pressure (1.5–20 bars) until sudden release of the pressure. Steam explosion thereby disrupts the cell wall structure and involves: (1) destruction of LCCs (Hongzhang and Liying 2007), (2) autohydrolysis of hemicelluloses, (3) delignification (Alvira et al. 2010; Kumar et al. 2009; Li et al. 2007; Sun et al. 2005) and under severe conditions (4) cellulose dissolution and hydrolysis (Huang et al. 2015; Jacquet et al. 2012; Sun et al. 2005). With such efficacy at opening wood structure, steam explosion has been a pretreatment of predilection for extracting nanocellulose from various lignocellulosic residues including most recently wood sawdust (Cherian et al. 2008, 2010, 2011; Deepa et al. 2011; Kalita et al. 2015). In these prior studies, a bleaching and acid hydrolysis steps were necessary to liberate the CNCs from the steam exploded lignocellulose. To date this pretreatment strategy has not been used in combination with [EMIM][OAc] for CNC extraction from lignocellulose.

This study aims at (1) reducing the severity of the [EMIM][OAc]-mediated CNC extraction by pretreating the wood using steam explosion and (2) validating the mechanistic interpretation reported earlier on the interaction between [EMIM][OAc] and wood structure and components.

Materials and methods

Materials

Angelim Vermelho (Dinizia *excelsa*) wood obtained from the Amazonian rain forests was milled to a particle size of 150 μm and dewaxed for 8 h using an ethanol:acetone:toluene mixture (1:1:4 volume ratio) in a soxhlet operating at 120 °C. The dewaxed wood flour (ca. 9 % mass loss) was then washed with hot water and oven-dried overnight at 60 °C under vacuum and kept in a vacuum desiccator until use.

1-ethyl-3-methylimidazolium acetate [EMIM][OAc], acetyl bromide, hydroxylamine hydrochloride, hydrogen peroxide, sodium hydroxide, and phenolphthalein were purchased from Sigma-Aldrich. Acetone, ethanol, toluene, dimethylsulfoxide (DMSO), acetic acid, and hydrochloric acid were purchased from VWR. Potassium bromide for IR was purchased from Merck.

Extraction of cellulose nanocrystals with steam explosion and [EMIM][OAc] treatment of wood

For the extraction of cellulose nanocrystals, the wood was pretreated using steam explosion before the [EMIM][OAc] treatment (Fig. 1). Namely, 4.0 g of Angelim Vermelho was soaked in a 400-mL beaker containing 100 mL of NaOH solution with a certain concentration [2 % (w/w) or 5 % (w/w)]. The beaker was covered using Aluminum foil and placed inside an autoclave (Systec, Germany) containing deionized water. The setup was heated to a set temperature (110 °C or 127 °C) to achieve a defined pressure (1.5 or 2.5 bar, respectively). Steaming was kept for a fixed period of time (1 or 2 h), after which the steam was released gradually (about 10–20 min). The steam-exploded wood mixture was quenched with 200 mL of deionized water. The steam-exploded Angelim Vermelho was directly filtered off (to remove the dissolved wood) and was washed continuously with water then oven-dried under vacuum overnight at 60 °C. Wood-0 and Wood-SE refer to the untreated and steam-exploded wood, respectively.

2.0 g of Wood-SE was stirred in open atmosphere with 40.0 g of [EMIM][OAc] pre-conditioned at 65 °C. After 1.5 h of mixing, the mixture was centrifuged at 12,000 rpm for 10 min (F156X100Y Multifuge, Thermo Scientific, USA), to separate the dissolved and undissolved wood, yielding a precipitate (undissolved

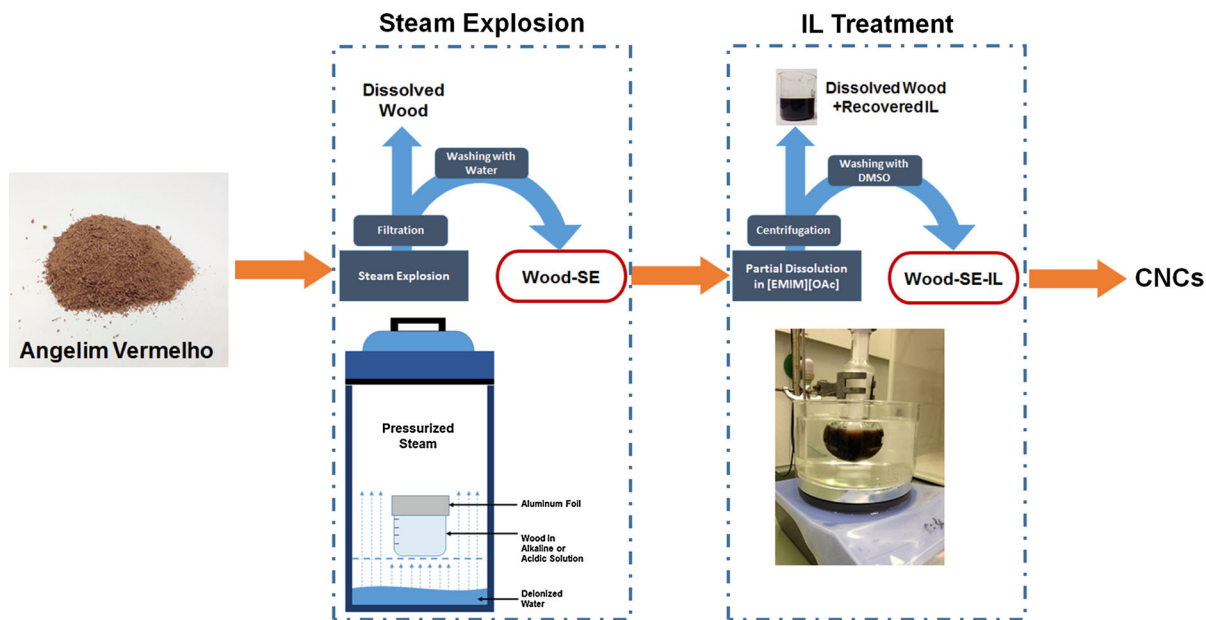


Fig. 1 Process for the extraction of cellulose nanocrystals directly from wood

wood) and a supernatant (dissolved wood + IL). The supernatant was decanted into a 200 mL Erlenmeyer flask. The precipitate was washed multiple times with DMSO and deionized water to remove any residual dissolved wood or residual ionic liquid. The residual lignin in the undissolved wood was removed by bleaching with 5 % H_2O_2 at 60 °C for 2 h, followed by washing with DI water. It was subsequently oven-dried at 60 °C under vacuum overnight and kept in a desiccator until characterization (Wood-SE-IL). Upon dispersing Wood-SE-IL in deionized water, and rapid sonication (30 s in ultrasonicator UW 2200, Bandelin Electronic, Germany), a turbid supernatant formed. The turbid supernatant was collected until no more turbidity was apparent, then subjected to further characterization as a dispersion and as a freeze-dried material. The steam explosion and [EMIM][OAc] treatment experiments were performed in duplicate to ascertain reproducibility. All reactants and wood residues were weighed using an analytical balance (Mettler-Toledo, Switzerland) with a precision of ± 0.01 mg.

Characterization methods

Wood chemical changes upon steam explosion and [EMIM][OAc] treatment were monitored with Fourier-Transform Infrared Spectroscopy (FT-IR) and

X-ray Diffraction (XRD). For FT-IR analysis, KBr pellets were formed by mixing 2 mg of sample with 200 mg of potassium bromide (KBr). Spectra were collected in transmittance mode on a FT-IR spectrometer 65 (Perkin Elmer, USA) using 64 scans at a resolution of 2 cm^{-1} . For XRD analysis, a Seifert 3003 TT X-ray diffractometer (General Electric, USA) using $\text{CuK}\alpha$ as a radiation source and operation at 40 kV and 30 mA was used in transmission mode. The intensities were collected at 2θ from 5 to 40. The crystalline structure was determined using Match! Software (version 1.11c) and the crystallinity index (CrI) was calculated according to Segal's method (Segal et al. 1959).

For a precise assessment of chemical changes upon both treatments, wood composition was determined using traditional wet chemistry approaches. Lignin content was measured with the method of Foster et al. (2010) with some modifications. The absorbance of the final solutions was determined on a UV spectrophotometer TIDAS (J&M Analytik AG, Germany). The lignin content was then calculated based on a UV calibration curve previously constructed with wood-milled lignin solutions of known concentrations. The wood-milled lignin was obtained from Angelim Vermelho by the method of Björkman. A specific absorptivity of 22.25 L g^{-1} was calculated from the

calibration curve (Björkman 1956). Holocellulose and cellulose contents were determined following the method of Ona et al. (1995). The cellulose obtained from the original wood (Wood-0) using this method (alkaline removal of hemicelluloses followed by bleaching) was used later as a reference for comparison purposes. It was referred to as original cellulose (CELL-0).

The collected turbid supernatant after SE and IL treatment was characterized for morphology, size distribution and surface properties with AFM, DSC, and contact angle. The characterization methods followed those of our previous publication (Abushamala et al. 2015). Briefly, for *Atomic Force Microscopy (AFM)*: a droplet of a diluted sample from the supernatant of Wood-SE-IL dispersion in water was allowed to dry overnight on a fresh surface of mica. The sample was then observed in tapping mode with an atomic force microscope (Nanoscope III) equipped with a tube scanner (Veeco Santa Barbara, USA) using silicon tips (PPP-NCH, Nanoandmore, Germany). The tips had a resonance frequency of 360 kHz and a spring constant of 50 N.m⁻¹. The particle dimensions (height and length) were analyzed using Nanoscope Analysis software (version 1.40) for a population of 100 particles.

The *degree of polymerization (DP)* of the wood original cellulose (CELL-0) and the extracted cellulose nanocrystals (CNCs) were determined on duplicate samples after carbanilation with phenyl isocyanate (Hubbell and Ragauskas 2010). The gel permeation chromatography system PSS 1200 (Agilent Technologies, USA) was equipped with three styragel columns (PSS SDV 5 µm: 102, 103, and 104) and ultraviolet (1200 UV 254 nm) and refractive index (Knauer K-2301) detectors with tetrahydrofuran as a mobile phase (flow rate: 1.0 mL/min). The injection volume was 50 µL and the column temperature was 30 °C. The calibration was performed using polystyrene standards (ReadyCal-Kit high, PSS-pskitr1h-04).

Degree of Acetylation (DA) was obtained with the back-titration method of Kim et al. (Kim et al. 2002).

Differential Scanning Calorimetry (DSC): about 10 mg sample of the cellulose nanocrystals (CNCs) was placed in differential scanning calorimeter DSC 8500 (Perkin Elmer, USA) and heated to 200 °C at 10 °C/min under nitrogen gas stream.

Contact Angle (CA) Measurement: the contact angle of the cellulose nanocrystals (CNCs) and the original cellulose (CELL-0) was measured using Digidrop equipment (GBX, France) by placing a water droplet volume of 4 µl on the surface followed by the required tangent procedure.

Results and discussion

Optimization of steam explosion pretreatment for Angelim Vermelho

The steam explosion process was optimized to achieve minimum cellulose degradation with maximum dissolution of lignin and hemicelluloses (Wu et al. 1999). NaOH concentration, steaming pressure (or temperature) and time were the main factors in the optimization while wood particle size and solid/liquid mixing ratio were kept constant (150 µm and 4 g/100 mL, respectively). Expectedly, NaOH presence during steam explosion facilitates the removal of hemicelluloses and lignin. With both 2 % and 5 % NaOH, most of the hemicelluloses and about 50 % of the lignin are removed (Table 1). At the same time crystallographic analysis of the steam exploded wood shows a 50 % increase in apparent crystallinity index, CrI, (Segal et al. 1959) and the preservation of the cellulose I microstructure viz. no mercerization (Fig. 5). Again, the 2 or 5 % NaOH solutions have similar impact on CrI in agreement with the chemical composition. Likewise, variations of steaming pressure and time did not have a significant impact on the chemical composition of the steam-exploded wood nor did the crystallinity index vary significantly (± 3 %). Based on these chemical and morphological analyses, the mildest condition for steam explosion, viz. treatment in 2 % NaOH concentration for 1 h under 1.5 bar was selected for further attempts at extracting cellulose nanocrystals with [EMIM][OAc].

Optimization of the [EMIM][OAc] treatment for CNC extraction

The steam-exploded wood (Wood-SE) containing approximately 25 % lignin and 75 % cellulose was subjected to [EMIM][OAc] treatment. With the previously published IL treatment conditions (65 °C and 1.5 h), the steam-exploded wood almost completely

Table 1 The chemical composition and crystallinity index (% CrI) of Angelim Vermelho upon steam explosion

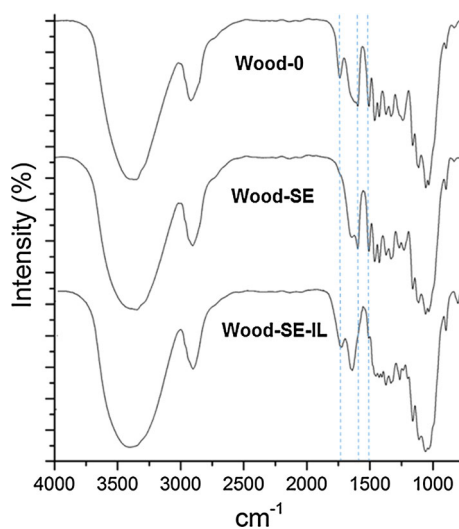
	SE conditions			Composition and crystallinity of Wood-SE			% CrI (%)
	NaOH (%)	P (bar)	t (h)	Cellulose (g)	Hemicelluloses (g)	Lignin (g)	
	–	–	–	47.6	19.4	33.0	41.5
	Water	2.5	2	47.4	16.8	31.9	42.9
	2	1.5	1	42.8	4.5	15.6	60.1
	2	1.5	2	45.4	3.0	15.1	59.8
	2	2.5	1	44.0	1.8	13.2	62.4
	2	2.5	2	45.4	1.4	14.7	61.2
The standard deviation was less than 3 % for all measurements. The chemical composition assumes 100 g of wood as a starting material	5	1.5	1	44.9	1.8	14.5	63.2
	5	1.5	2	46.9	2.3	14.1	61.2
	5	2.5	1	46.4	0.8	15.5	62.7
	5	2.5	2	46.9	1.8	14.7	62.1

Table 2 The mass recovery (% MR) and crystallinity index (% CrI) of the steam-exploded wood after the IL treatment under various temperature and time conditions

T (°C)	t (min)	MR (%)	% CrI (%)
65	90	3.9 ± 1.6	–
60	60	6.1 ± 2.7	–
50	60	21.8 ± 2.9	29.7 ± 4.2
40	30	43.8 ± 2.4	52.7 ± 3.3
30	15	77.1 ± 4.3	73.1 ± 2.7

dissolved (Table 2). Such severe dissolution is consistent with the expectation that steam-exploded wood is more vulnerable to chemical treatment (Grous et al. 1986; Hongzhang and Liying 2007). Milder conditions were thus explored to reduce the dissolution extent in the IL cycle while monitoring the crystallinity of the wood residue (Wood-SE-IL) (Table 2). By successively decreasing the treatment time, a very mild IL treatment at 30 °C for 15 min was found to allow the recovery of a significant amount of the wood residue. The wood residue was more than 90 % rich in cellulose and maintained a cellulose I microstructure with an increased apparent crystallinity index of 73.1 % (± 2.7). The steam exploded and IL-treated wood under optimized conditions (Wood-SE-IL) was then bleached using hydrogen peroxide, which did not change its CrI (73.3 % ± 1.6) but induced a small mass loss (8.2 %) ascribed to lignin removal.

FT-IR confirmed the chemical changes induced by the optimized steam explosion and IL treatment (Fig. 2). In contrast to the raw wood sample (Wood-0), the steam exploded wood (Wood-SE) had no

**Fig. 2** The FT-IR spectra of the optimum wood residues after steam explosion (Wood-SE) and IL treatment (Wood-SE-IL) compared to the original wood (Wood-0)

detectable carbonyl vibration (1740 cm^{-1}), confirming autohydrolysis of hemicelluloses upon steam explosion (Table 1). Minor drop in the intensity of the phenolics fingerprint at 1510 cm^{-1} and 1610 cm^{-1} was observed although ca. 50 % delignification was evident upon steam explosion (Table 1). After IL treatment and the peroxide-based bleaching, almost complete delignification was evidenced by the quasi-absence of phenolics fingerprints (1510 and 1610 cm^{-1}). Additionally, acetylation by [EMIM][OAc] was evident as repeatedly reported (Abushammala et al. 2015; Çetinkol et al. 2010; Ebner et al. 2008; Hauru et al. 2013; Karatzos et al. 2012; King et al. 2014; Köhler et al. 2007).

The pulp after steam explosion and the IL treatment with [EMIM][OAc] (Wood-SE-IL) was highly dispersible in water. The dispersion was allowed to sediment and a turbid supernatant was collected and further analyzed. 87.4 % of Wood-SE-IL was collected in the form of suspension. This represents ca. 52.7 % of the cellulose content in the native wood.

Characterization of the CNCs in the supernatant

AFM confirmed the presence of CNCs in the supernatant (Fig. 3). The x-ray diffraction pattern of the freeze-dried supernatant confirms the native cellulose crystalline structure with the characteristic convoluted diffraction peaks at 2θ equal to 14.8° , 16.3° , 20.4° , 22.4° and 34.5° , which correspond to the crystallographic planes $[-110]$, $[110]$, $[102]$, $[200]$ and $[004]$, respectively. Cellulose II crystalline structure is not observed at 2θ equal to 12° . Crystallinity index

increased from ca. 41.5 ± 1.0 % for the native wood to 75.6 ± 1.0 % for the nanocrystals of the Wood-SE-IL. This morphological analysis confirmed that native cellulose nanocrystals are also easily extracted from mildly steam exploded wood with the IL approach. Based on the particle size distribution obtained from the AFM image (Fig. 3), the nanocrystals were about 2.6 nm in width and about 225 nm in length. These nanocrystals are evidently thinner than those usually obtained from pulp and microcrystalline cellulose since cellulose crystallites thicken during pulping and acid hydrolysis (Leppänen et al. 2009).

These dimensions are consistent with the degree of polymerization of the CNCs measured at 394, viz. close to the level-off degree of polymerization of cellulose (Battista et al. 1956), and one order of magnitude lower than that of the original cellulose in wood at 3016. Cellulose hydrolysis has been reported to take place during steam explosion (Jacquet et al.

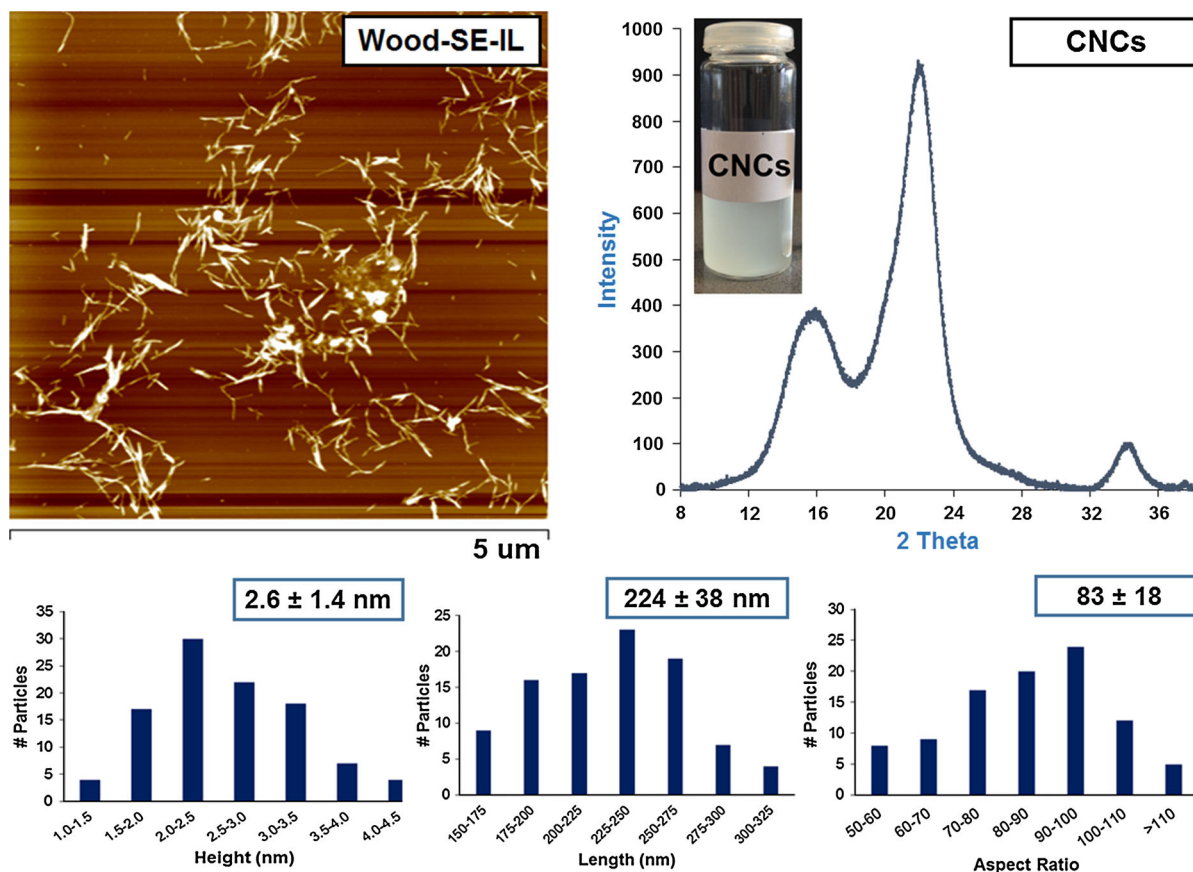


Fig. 3 AFM height image and WAXS diffractogram for the supernatant of the pulp dispersion (Wood-SE-IL) with size distribution of the corresponding nanocrystals

2012; Joshi et al. 2011; Sun et al. 2005) and [EMIM][OAc] treatment (Abushammala et al. 2015; Gazit and Katz 2012), separately. Cellulose hydrolysis using [EMIM][OAc] has been ascribed to the action of the proton generated from the imidazolium ring (Ebner et al. 2008; Gazit and Katz 2012). Molecular weight and X-ray diffraction results confirm that cellulose hydrolysis has taken place mainly in cellulose amorphous regions and keeping crystallites intact.

Acetylation is now well established during [EMIM][OAc] treatment of wood and cellulose and was again suggested by FT-IR in this study (Fig. 2). A more quantitative assessment of DA with back-titration method (Kim et al. 2002), revealed a DA of 0.46 (or 15.3 %). The measured acetylation led to an overestimation of the cellulose mass after the ionic liquid treatment (ca. 10 % increase in mass). Therefore, the corrected yield for nanocrystals based on the initial wood cellulose content becomes 44.7 % (instead of 52.7 %). As previously shown, secondary evidence of this partial acetylation of the CNCs can be found in the detection of a glass transition temperature at 107 °C (Fig. 6) and the increase in contact angle from 42° for pure cellulose to 67° for CNCs (Fig. 7) (Sakellariou et al. 1985).

Comparison and discussion of the SE/IL procedure and the double-cycle ionic liquid (IL/IL) procedure

To this stage, it is clearly established that combining steam explosion with a mild IL treatment is efficient at liberating cellulose nanocrystals from wood. Comparing the present results with the initial report, in which wood was treated with the same IL under harsher conditions twice (65, 1 h) in order to liberate CNCs, several observations are in order. In the SE/IL route, the steam explosion step allowed a milder IL treatment step (30 °C for only 15 min). The severity of each route can be quantified with a severity factor according to (Overend et al. 1987):

$$\text{Severity Factor}(SF) = \log_{10} \left[t \times e^{\left[\frac{T-100}{14.75} \right]} \right]$$

where t is the time of the process (in min), T is the applied temperature.

In the IL/IL route, the total severity factor is 1.84 while in the SE/IL route an overall lower severity factor of overall severity factor of 1.40 is obtained. While the steam explosion has a high severity factor of (2.28), the subsequent IL cycle is so mild that it has a

negative severity factor of (−0.88). Thus the SE/IL route appears overall milder than the route exclusively relying on the ionic liquid use. Caution must however be exerted in strictly comparing these numbers, as pointed out by (Pedersen and Meyer 2010). The multiple factors affecting the opening of the lignocellulosic matrix largely complicates its modeling resulting in unreliable calculations of severity factors. In all cases it is clear that after the steam explosion treatment, it takes a short immersion of steam-exploded biomass into the ionic liquid under near ambient conditions to deliver the CNCs.

The yield provides another indication of the efficacy of the proposed route. The original IL/IL route has a higher CNC yield (58 %) based on the cellulose content of wood. In the case of the SE/IL route, the yield decreases to approximately 45 % of wood initial cellulose content. In other words, in this case there is a small loss of cellulose native crystals in the process. Overall both processes deliver CNCs in higher yields than those obtained with the traditional approaches of pulping and acid hydrolysis. Another advantage of the process is that the collection of CNCs in both cycles is done through liquid/liquid extraction thus circumventing the need for acid neutralization and CNC purification via the labor intensive and time consuming centrifugation/dialysis process. With both SE/IL and IL/IL routes the elimination of this bottleneck step significantly simplifies the entire production process and reduces production time.

Also interesting is the quality of the CNCs obtained from the SE/IL route in terms of dimensions, morphology and chemical attributes (Table 3). The resulting CNCs are thinner (2.6 ± 1.4 nm) and somewhat longer (224 ± 38) than CNCs obtained by the IL/IL method (aspect ratio ~ 65) or by the sulfuric acid hydrolysis method from pulp (aspect ratio ~ 20 – 40) (Beck-Candanedo et al. 2005; Hamad and Hu 2010). The SE/IL CNCs thus display remarkable aspect ratios of 83 ± 18 . This aspect ratio is well over that reported previously for any other wood sources or even tunicate sources. In wood, the biosynthesis process results in cellulose I fibrils with lateral dimensions of 3–4 nm, which are further assembled into 10–30 nm wide aggregates forming a lenticular structure (Boyd 1982; Larsson et al. 1997; Salmén and Burgert 2009; Wickholm et al. 1998). The surface of these fibrils is coated with wood amorphous polymers (lignin and hemicelluloses) in a rather organized fashion

Table 3 Comparison of the yield and properties of the extracted CNCs from the two procedures; IL/IL and SE/IL

Process	IL/IL	SE/IL
1st cycle	65 °C, 1.5 h	1.5 bar, 1 h, 2 %
2nd cycle	65 °C, 1.5 h	30 °C, 15 min
SF	1.84	1.40
Yield (%)	58.2	44.7
% CrI (%)	76.2	75.6
L (nm)	181 ± 41	224 ± 38
D (nm)	3.2 ± 1.4	2.6 ± 1.4
L/D	64 ± 28	83 ± 18
DS	0.39 ± 0.08	0.46 ± 0.11
CA	57° ± 5	67° ± 4
T _g	116 °C ± 12	107 °C ± 9

SF, severity factor; L, length; D, lateral dimension; L/D, aspect ratio; DS, acetylation degree; CA, contact angle

(Terashima et al. 2009). During pulping, cellulose fibril aggregation further occurs as a consequence of a loss of wood amorphous polymers, which otherwise play the role of restricting spacers among the fibrils (Duchesne and Daniel 2000; Duchesne et al. 2001; Fahlén and Salmén 2003; Hult et al. 2001; Molin and Teder 2002). It results that CNCs extracted from pulp have crystallite lateral dimensions larger than those in wood. In contrast, when extracting cellulose crystallites directly from wood with IL, some residual lignin and hemicelluloses might remain as spacer within the cellulose lenticular structure. As a result, cellulose crystallite aggregation and/or co-crystallization is likely inhibited by the residual polymers from wood amorphous matrix. Indeed the raw CNCs obtained in the supernatant directly after IL treatment (without bleaching) are clearly still coated with lignin as evidenced by their brown aspect (Fig. 4). Therefore, the observations that the CNCs extracted directly from wood have smaller lateral dimensions, exceptional high aspect ratios and are still covered with lignin are all consistent.

We also note that the overall crystallinity of 76 % is slightly lower than that obtained from pulp based CNCs (~80–90 %). However, the produced CNCs have comparable crystallinity index than that of CNCs extracted using the IL/IL route (Table 3). The acetylation degree value obtained by the SE/IL route is higher (DS = 0.49) although the severity of the IL step in this route has been minimized significantly (30 °C—15 min) (Table 3). As steam explosion increases the



Fig. 4 AFM height image and photograph of unbleached CNCs obtained from SE/IL treatment demonstrating its color in the presence of lignin

accessibility to wood polymers, acetylation might be facilitated (Grous et al. 1986). As observed before with the IL/IL treatment, the increased acetylation led to a decrease in the glass transition temperature of the nanocrystals and increase in the contact angle (Table 3; Figs. 6, 7).

Conclusions

We demonstrate a new CNC extraction process combining a mild steam explosion treatment with immersion in 1-ethyl-3-methylimidazolium acetate for only 15 min at 30 °C. This process allows the recovery of CNCs in ca. 45 % yield (based on wood cellulose content) directly from wood and with exceptional aspect ratios in the 80–85 range. The CNCs were acetylated and exhibited residual lignin on their surface. These surface modifications are proposed to play a role of spacers in cellulose lenticular structure allowing to maintain the thin lateral dimensions (2.6 ± 1.4 nm) of native cellulose in wood. The high aspect ratios of the CNCs together with the ease of the process—viz. no purification/dialysis required—is particularly attractive for direct value-adding to lignocellulosic biomass into cellulose nanocrystals. Further efforts are ongoing to widen this process to a variety of lignocellulosic

biomass and assess the hypothesis that CNCs can thereby be collected in their native crystallite sizes.

Acknowledgments This research was funded by German Academic Exchange Service (DAAD), German Society of International Collaboration (GIZ) and Brazilian Department of higher Education (CAPES program) through the Novas Parcerias (NoPa) program for Brazilian-German academic exchanges. The authors thank Elke Stibal for the technical support, Adrian Vogt and Sebastian Schutt for the XRD support, Marina Hagios for GPC measurements, and Dr. Leif Nutto and ORSA Company for the Angelim Vermelho sample.

Appendix

See Figs. 5, 6 and 7.

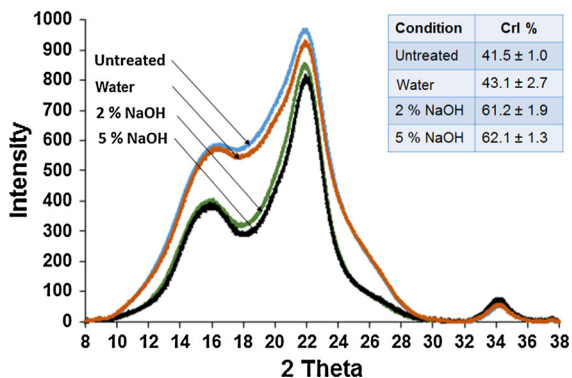


Fig. 5 The X-ray diffraction pattern of Angelim Vermelho upon steam explosion (2.5 bar, 2 h) at varying NaOH concentration compared to the untreated one

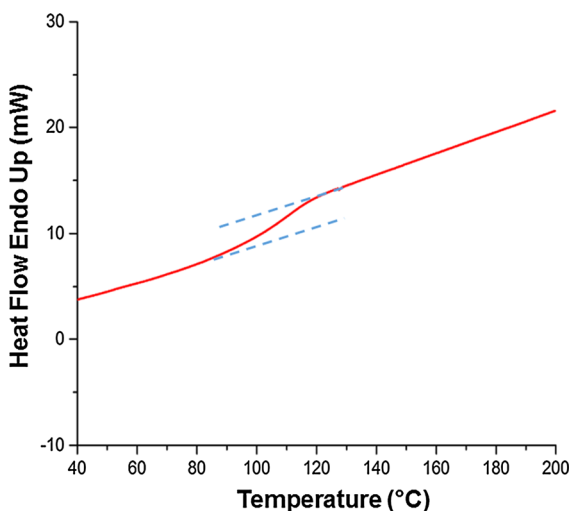


Fig. 6 DSC of the cellulose nanocrystals (CNCs). A glass transition temperature at 107 °C was detected due to acetylation

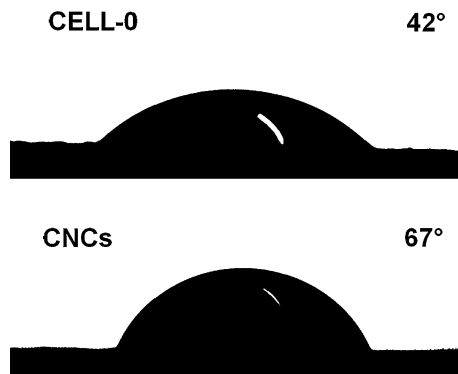


Fig. 7 The contact angle of the cellulose nanocrystals (CNCs) compared to the original cellulose (CELL-0)

References

- Abushammala H, Krossing I, Laborie M-P (2015) Ionic liquid-mediated technology to produce cellulose nanocrystals directly from wood. *Carbohydr Polym* 134:609–616
- Alvira P, Tomás-Pejó E, Ballesteros M, Negro M (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour Technol* 101:4851–4861
- Battista O, Coppick S, Howsmon J, Morehead F, Sisson WA (1956) Level-off degree of polymerization. *Ind Eng Chem* 48:333–335
- Beck-Candanedo S, Roman M, Gray DG (2005) Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. *Biomacromolecules* 6:1048–1054
- Björkman A (1956) Studies on finely divided wood. Part 1. Extraction of lignin with neutral solvents. *Svensk Papperstidning* 59:477–485
- Bondeson D, Mathew A, Oksman K (2006) Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis. *Cellulose* 13:171–180
- Boyd J (1982) An anatomical explanation for visco-elastic and mechano-sorptive creep in wood, and effects of loading rate on strength. In: Bass P (ed) *New perspectives in wood anatomy*. Springer, New York, pp 171–222
- Çetinkol ÖP et al (2010) Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* 1:33–46
- Cherian BM, Pothan LA, Nguyen-Chung T, Mennig G, Kottaisamy M, Thomas S (2008) A novel method for the synthesis of cellulose nanofibril whiskers from banana fibers and characterization. *J Agric Food Chem* 56:5617–5627. doi:10.1021/jf8003674
- Cherian BM, Leão AL, de Souza SF, Thomas S, Pothan LA, Kottaisamy M (2010) Isolation of nanocellulose from pineapple leaf fibres by steam explosion. *Carbohydr Polym* 81:720–725
- Cherian BM et al (2011) Cellulose nanocomposites with nanofibers isolated from pineapple leaf fibers for medical applications. *Carbohydr Polym* 96:1790–1798

- Deepa B et al (2011) Structure, morphology and thermal characteristics of banana nano fibers obtained by steam explosion. *Bioresour Technol* 102:1988–1997
- Dong H, Strawhecker KE, Snyder JF, Orlicki JA, Reiner RS, Rudie AW (2012) Cellulose nanocrystals as a reinforcing material for electrospun poly(methyl methacrylate) fibers: formation, properties and nanomechanical characterization. *Carbohydr Polym* 87:2488–2495
- Duchesne I, Daniel G (2000) Changes in surface ultrastructure of Norway spruce fibres during kraft pulping-visualisation by field emission-SEM. *Nord Pulp Pap Res J* 15:54–61
- Duchesne I, Hult E, Molin U, Daniel G, Iversen T, Lennholm H (2001) The influence of hemicellulose on fibril aggregation of kraft pulp fibres as revealed by FE-SEM and CP/MAS 13C-NMR. *Cellulose* 8:103–111
- Ebner G, Schiehsler S, Potthast A, Rosenau T (2008) Side reaction of cellulose with common 1-alkyl-3-methylimidazolium-based ionic liquids. *Tetrahedron Lett* 49:7322–7324
- Fahlén J, Salmén L (2003) Cross-sectional structure of the secondary wall of wood fibers as affected by processing. *J Mater Sci* 38:119–126
- Foster CE, Martin TM, Pauly M (2010) Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part I. Lignin J Vis Exp JoVE 37:1745
- Gazit OM, Katz A (2012) Dialkylimidazolium ionic liquids hydrolyze cellulose under mild conditions. *ACS Sustain Chem Eng* 5:1542–1548
- Grous WR, Converse AO, Grethlein HE (1986) Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar. *Enzyme Microb Technol* 8:274–280
- Hamad WY, Hu TQ (2010) Structure–process–yield interrelations in nanocrystalline cellulose extraction. *Can J Chem Eng* 88:392–402
- Hauru LK et al (2013) Enhancement of ionic liquid-aided fractionation of birchwood. Part 1: autohydrolysis pretreatment. *RSC Adv* 3:16365–16373
- Hongzhang C, Liying L (2007) Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. *Bioresour Technol* 98:666–676
- Huang Y et al (2015) Steam explosion distinctively enhances biomass enzymatic saccharification of cotton stalks by largely reducing cellulose polymerization degree in *G. barbadense* and *G. hirsutum*. *Bioresour Technol* 181:224–230
- Hubbell CA, Ragauskas AJ (2010) Effect of acid-chlorite delignification on cellulose degree of polymerization. *Bioresour Technol* 101:7410–7415
- Hult E-L, Larsson P, Iversen T (2001) Cellulose fibril aggregation—an inherent property of kraft pulps. *Polymer* 42:3309–3314
- Jacquet N, Vanderghem C, Danthine S, Quiévy N, Blecker C, Devaux J, Paquot M (2012) Influence of steam explosion on physicochemical properties and hydrolysis rate of pure cellulose fibers. *Bioresour Technol* 121:221–227
- Joshi B, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L (2011) Lignocellulosic ethanol production: current practices and recent developments. *Biotechnol Mol Biol Rev* 6:172–182
- Kalita E, Nath B, Deb P, Agan F, Islam MR, Saikia K (2015) High quality fluorescent cellulose nanofibers from endemic rice husk: isolation and characterization. *Carbohydr Polym* 122:308–313
- Karatzos SK, Edey LA, Wellard RM (2012) The undesirable acetylation of cellulose by the acetate ion of 1-ethyl-3-methylimidazolium acetate. *Cellulose* 19:307–312
- Kaushik A, Singh M, Verma G (2010) Green nanocomposites based on thermoplastic starch and steam exploded cellulose nanofibrils from wheat straw. *Carbohydr Polym* 82:337–345
- Kim D-Y, Nishiyama Y, Kuga S (2002) Surface acetylation of bacterial cellulose. *Cellulose* 9:361–367
- King A, Xie H, Fiskari J, Kilpeläinen I (2014) Reduction of biomass recalcitrance via ionic liquid pretreatments. In: Ragauskas AJ (ed) *Materials for biofuels*. World Scientific Publishing Company, Singapore, pp 95–125
- Köhler S, Liebert T, Schöbitz M, Schaller J, Meister F, Günther W, Heinze T (2007) Interactions of ionic liquids with polysaccharides 1. Unexpected acetylation of cellulose with 1-ethyl-3-methylimidazolium acetate. *Macromol Rapid Commun* 28:2311–2317
- Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res* 48:3713–3729
- Larsson PT, Wickholm K, Iversen T (1997) A CP/MAS 13 C NMR investigation of molecular ordering in celluloses. *Carbohydr Res* 302:19–25
- Leppänen K, Andersson S, Torkkeli M, Knaapila M, Kotelnikova N, Serimaa R (2009) Structure of cellulose and microcrystalline cellulose from various wood species, cotton and flax studied by X-ray scattering. *Cellulose* 16:999–1015
- Li J, Henriksson G, Gellerstedt G (2007) Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour Technol* 98:3061–3068
- Mason WH (1928) US Patent 1655618 A. <https://www.google.com/patents/US1655618>
- Molin U, Teder A (2002) Importance of cellulose/hemicellulose-ratio for pulp strength. *Nord Pulp Pap Res J* 17:14
- Ona T, Sonoda T, Shibata M, Fukazawa K (1995) Small-scale method to determine the content of wood components from multiple eucalypt samples. *Tappi J* 78:121–126
- Overend RP, Chornet E, Gascoigne J (1987) Fractionation of lignocellulosics by steam-aqueous pretreatments [and discussion]. *Philos Trans R Soc Lond A Math Phys Eng Sci* 321:523–536
- Pedersen M, Meyer AS (2010) Lignocellulose pretreatment severity—relating pH to biomatrix opening. *New Biotechnol* 27:739–750
- Ruiz E, Cara C, Manzanares P, Ballesteros M, Castro E (2008) Evaluation of steam explosion pre-treatment for enzymatic hydrolysis of sunflower stalks. *Enzyme Microb Technol* 42:160–166
- Sakellariou P, Rowe R, White E (1985) The thermomechanical properties and glass transition temperatures of some cellulose derivatives used in film coating. *Int J Pharm* 27:267–277
- Salmén L, Burgert I (2009) Cell wall features with regard to mechanical performance. A review COST Action E35

- 2004–2008: wood machining—micromechanics and fracture. *Holzforschung* 63:121–129
- Segal L, Creely J, Martin A, Conrad C (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Text Res J* 29:786–794
- Sun XF, Xu F, Sun RC, Fowler P, Baird MS (2005) Characteristics of degraded cellulose obtained from steam-exploded wheat straw. *Carbohydr Res* 340:97–106
- Terashima N, Kitano K, Kojima M, Yoshida M, Yamamoto H, Westermark U (2009) Nanostructural assembly of cellulose, hemicellulose, and lignin in the middle layer of secondary wall of ginkgo tracheid. *J Wood Sci* 55:409–416
- Wickholm K, Larsson PT, Iversen T (1998) Assignment of non-crystalline forms in cellulose I by CP/MAS ^{13}C NMR spectroscopy. *Carbohydr Res* 312:123–129
- Wu M, Chang K, Gregg D, Boussaid A, Beatson R, Saddler J (1999) Optimization of steam explosion to enhance hemicellulose recovery and enzymatic hydrolysis of cellulose in softwoods. *Appl Biochem Biotechnol* 77:47–54
- Zweckmair T, Hettegger H, Abushammala H, Bacher M, Potthast A, Laborie M-P, Rosenau T (2015) On the mechanism of the unwanted acetylation of polysaccharides by 1, 3-dialkylimidazolium acetate ionic liquids: part 1—analysis, acetylating agent, influence of water, and mechanistic considerations. *Cellulose* 22:3583–3596