

Fractionation of Lignocellulosic Materials Using Ionic Liquids: Part 2. Effect of Particle Size on the Mechanisms of Fractionation

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S Supporting Information

ABSTRACT: In part 1 of this effort (*Ind. Eng. Chem. Res.* **2011**, *50*, 12349–12357), we studied how wood dissolved in ionic liquid (IL) is precipitated into different molecular weight ranges upon the addition of a cosolvent. In this article, we further analyze the chemical compositions of these fractions and elucidate the mechanisms of fractionation. Specifically, we fractionated Norway spruce wood solvated with 1-allyl-3-methylimidazolium chloride ([amim]Cl) and analyzed the resulting fractions by Klason lignin analysis and FT-IR and NMR spectroscopies. We found that separation of the different components can be tuned by the variable dissolution of wood based on particle size, resulting from preparatory milling. It is possible to obtain cellulose-rich material with a relatively low (6.2%) lignin content, from spruce sawdust. This can be achieved by extracting the cellulose from the insoluble lignin–carbohydrate complex (LCC) matrix. Extensive milling of wood afforded a soluble LCC matrix, and its precipitation was based on molecular weight and not on chemical composition. Indications of the presence of LCCs in the hemicellulose fraction were obtained by utilizing multidimensional NMR spectroscopy.

■ INTRODUCTION

Lignocellulosic materials are a reasonable carbon-neutral option for the production of energy and materials in the future. Agricultural and forest residues and dedicated energy crops could be utilized in the production of biofuels and industrial chemicals,^{1–4} in addition to the manufacturing of novel polymeric^{5–7} and composite⁸ materials. In this article, we focus on wood, which is an abundant lignocellulosic feedstock. Wood is composed of three main polymeric components: cellulose, lignin, and hemicelluloses. Cellulose, the most abundant, is a linear polymer consisting of glucose units joined together by 1,4-glycosidic linkages. Because of the intramolecular hydrogen bonding between hydroxyl groups in the 2, 3, and 6 positions of the glucose units, these molecules are rigid and tend to arrange in layered structures. Intermolecular O3–O6 hydrogen bonds fasten the cellulose chains together into microfibrils that show both crystalline and amorphous domains.⁹ Lignin is a highly branched heterogeneous polymer that is built of phenylpropanoid units, linked with various types of ether and carbon–carbon bonds.¹⁰ Hemicelluloses are branched heteropolysaccharides. Two of the main hemicelluloses in softwood, on which we focus in our work, are galactoglucomannan and arabinan-4-O-methylglucuronoxylan. Their main structural units are mannose and xylose, respectively. Some of the backbone saccharide hydroxyl groups are functionalized as acetyls and glucuronic acid esters and with monosaccharide units.⁹ Together with lignin, they are thought to form a network structure in which lignin and hemicelluloses are bonded by benzyl ether, benzyl ester, and phenyl glycoside linkages.¹¹ These networks are clustered into parallel regions between the cellulose fibrils acting as a composite matrix.¹²

Ionic liquids (ILs) have been recognized as a promising way to fulfill goals in the utilization of woody biomass.^{13–15} In recent years, several groups have attempted the separation of wood components with ILs. Regardless of the different approaches, their efficient separation has turned out to be a demanding task. The main approaches include the selective precipitation of materials dissolved in an IL by nonsolvent addition¹⁶ or the selective extraction of one of the main components with the IL and precipitation of the extracted fraction.^{17–19} A combination of these techniques seems promising, based on the fact that Sun et al.²⁰ reported a more selective separation of carbohydrates when the whole wood starting material was not completely dissolved but was removed from the IL prior to the solvent precipitation step.

One potential limiting factor hindering the efficient separation of polysaccharides from lignin is the occurrence of lignin–carbohydrate complexes (LCCs). Recent studies carried out simultaneously with our work strongly support such a contention. Conditions able to physically alter or depolymerize lignin, such as high temperatures above its glass transition,²¹ oxidizing agents,²² and acid formation through autocatalytic processes, combined with an IL treatment have been found to enhance the cellulose enrichment in the regenerated materials. Therefore, a sound scientific understanding of the orientation of the wood components within the cell-wall structures is needed. In addition, knowledge related to the actual type and

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degree of covalent bonding between the lignin and polysaccharides is required. For hemicelluloses and lignin, the existence of LCCs is reasonably well documented for isolated materials.^{23–29} However, the occurrence of native hemicellulose–lignin and isolated cellulose–lignin complexes is rather controversial.^{25,28,30–32} Atalla et al.³³ published evidence that hemicelluloses, especially glucomannans,³⁴ are incorporated into cellulose fibrils,³³ which furthermore increase the physical resistance to dissolution and separation, because parts of each component are at least physically entangled, if not covalently bonded, with each other.

Sjöholm et al.³⁵ determined that lignin is subdivided into different molecular weight ranges in softwood and hardwood pulps. They found softwood lignin to be of a higher molecular weight and hardwood lignin of a lower molecular weight. This type of knowledge about the molecular weight distributions, morphologies, and linkages between different components in native and processed woods represents crucial understanding for their successful separation by precipitation. Understanding the chemical changes during IL treatment is also significant. Recently, we presented data³⁶ that further indicate that the precipitation order of different components of Norway spruce is dependent on the molecular weight of the precipitated fraction and not its composition. The relative contribution of the molecular weight distribution and the chemical composition to the selectivity of fractionation, however, might vary from species to species and with the degree of pretreatment.

In this article, we focus on compositional analyses of the fractions that were obtained by precipitating wood dissolved in [amim]Cl, using acetonitrile (MeCN), water, and methanol as nonsolvents or extraction/washing solvents. The fractions were analyzed by Klason lignin determination and FT-IR spectroscopy. In addition, two- and three-dimensional NMR spectroscopy was applied for further structural analyses of the fractions of mixed polysaccharides and lignin compositions. As a result, we found that different fractions were enriched in either cellulose, lignin, or hemicelluloses and that the data were highly dependent on both the mode of preparation of the starting material and the wood species. We also performed size-exclusion chromatography (SEC) measurements on these samples,³⁶ and by correlating these data with their compositional analyses, we arrived at conclusions related to the way wood components are precipitated.

EXPERIMENTAL SECTION

Materials. Acetonitrile (MeCN), allyl chloride, ethanol (EtOH), methanol (MeOH), 1-methylimidazole, and pyridine (Pyr) were purchased from Sigma-Aldrich. Allyl chloride and 1-methylimidazole were distilled prior to use. Unbleached Norway spruce (*Picea abies*) thermomechanical pulp (TMP) was donated from a Swedish mill. Norway spruce sawdust (particle size 0.1–1.0 mm) was prepared in-house with a belt grinder (grade 60). Prior to ball-milling treatments, Norway spruce TMP was first milled in a Wiley mill with a 20-mesh (0.84-mm) sieving screen. After Wiley milling, the 20-mesh powder was extracted in a Soxhlet extractor for 48 h with acetone. A portion of this fibrous material was further sieved to pass a coarse 40-mesh (0.40-mm) sieve. The remaining extracted 20-mesh Norway spruce powder was milled with a rotary ball mill in a ceramic-plated 5.5-L steel jar with 470 ceramic balls (diameter 0.9 cm) and a rotation speed of 60 rpm for 28 days. After milling, the fine powder was dried in a vacuum oven. The average particle size was determined to be

less than 200 mesh (75 μm). All of the wood materials were dried in a vacuum oven overnight at 40 °C prior to use.

Synthesis of [amim]Cl. Synthesis of [amim]Cl was performed according to a method adapted from Wu et al.³⁷ A Detailed description of the synthesis and purification steps can be found in our earlier publication.³⁶

Solvation of Wood with [amim]Cl. Lignocellulosic material (typically ca. 1 g) was quickly added to a flask containing dry [amim]Cl (typically ca. 20 g) under a nitrogen atmosphere. The mixture was homogenized with a vortex mixer until an even dispersion was obtained. Dissolution was performed in a temperature-controlled oil bath using a three-necked flask under positive pressure of nitrogen. This apparatus was equipped with an overhead mechanical stirrer with a steel blade. A positive pressure of nitrogen gas was maintained during the whole dissolution period. Solvation conditions and quantities of material were varied according to each experiment performed. Rotary-ball-milled Norway spruce powder generally dispersed and gave a clear solution in a short period of time. Mixtures containing Wiley-milled and sawdust materials remained slightly cloudy even after extensive heating at 100 or 110 °C.

Fractionation of Solvated Wood by Nonsolvent Addition. Preparation of fractions 1–4 was carried out using a method described in detail in the context of our earlier publication (Figure 1).³⁶

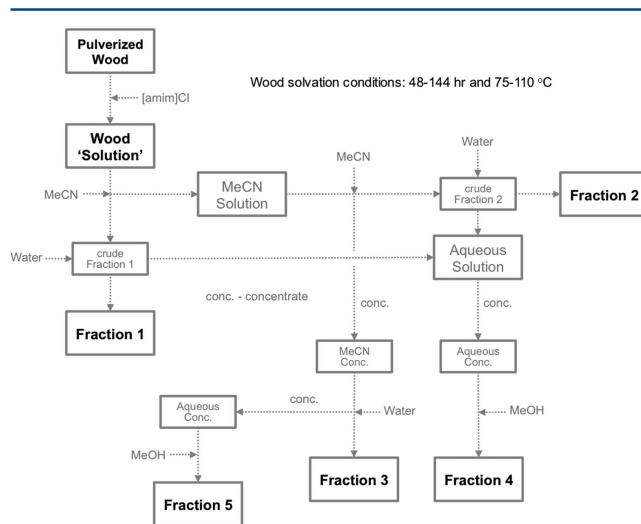


Figure 1. Total fractionation process described in detail in the Experimental Section. Fraction 5 was not collected from every solution.

Analysis of Precipitated Fractions. For molecular weight determination the fractionated samples were derivatized and analyzed using an HP G1312A pump connected to two Waters HR5E and HR1 Styragel columns equipped with a Waters 484 UV-absorbance detector (set at 280 nm) calibrated using polystyrene standards as described in our earlier work.³⁶ Acid-insoluble (Klason) lignin and acid-soluble lignin were determined according to procedures published by Dence et al.³⁸ The acid that was used to hydrolyze the samples was diluted, and the sulfuric acid concentration was verified by density determinations. The density of the diluted acid was measured using a hydrometer to be 1.6340 g/cm³, corresponding to 72% \pm 0.1%. The lignocellulose samples were dried in a vacuum oven at 40 °C overnight. Approximately 100 mg of

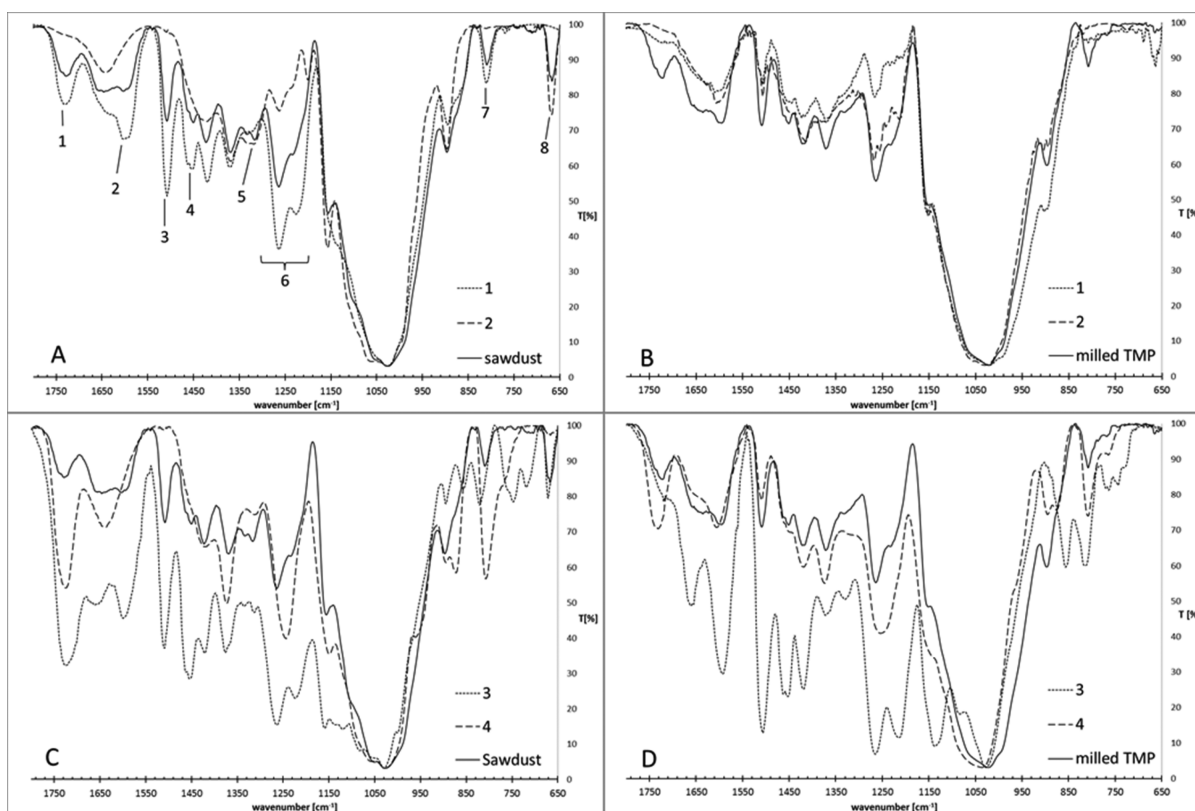


Figure 2. FT-IR spectra of fractions 1–4 precipitated with identified bands from solutions of sawdust and milled TMP compared to the starting materials: (A) sawdust fractions 1 and 2, (B) milled TMP fractions 1 and 2, (C) sawdust fractions 3 and 4, and (D) milled TMP fractions 3 and 4. Band assignments (see the text for references): (1) carbonyl from hemicelluloses and lignin, (2) carboxylic acid from xylan and lignin, (3) lignin, (4) xylan, (5) cellulose, (6) carbonyl from hemicelluloses and lignin, (7) glucumannan, and (8) cellulose.

each sample was measured accurately and mixed with sulfuric acid solution (100 mg per 2 mL) using magnetic stirring and a vortex mixer. After 2 h of hydrolysis at room temperature with occasional manual mixing, the samples were diluted with 50 mL of deionized water and transferred into sealable bottles. The bottles were placed into a commercial pressure cooker and heated at elevated pressure for 90 min. The solid residues were filtered with a grade-3 sinter and washed with 40 mL of water. The filtrate was retained for acid-soluble lignin determination. The solid residue was further washed with 60 mL of water, so that the filtrate was neutral, and after air-drying, the sample was placed into a vacuum oven for 20 h. Acid-soluble lignin was determined spectrophotometrically from the retained filtrates. The filtrates were first diluted to precisely 100 mL, and then the absorbance was measured at 205 nm wavelength in a 1 cm-path-length cuvette. The concentrations were calculated using an extinction coefficient of 110 L/(g cm).

FT-IR spectra were recorded from finely powdered samples that were dried for 20 h at 50 °C in a vacuum oven, using a Perkin-Elmer Spectrum One AT-IR spectrometer. Processing was carried out using PE Spectrum One software. The spectra were processed with baseline correction, noise elimination, and normalization.

For multidimensional NMR analyses (presented in the Supporting Information), a sample from fraction 4 was dissolved in deuterated dimethyl sulfoxide (DMSO- d_6 , 50 mg/mL). The chemical shifts were referenced to the residual DMSO signal (2.54/40.45 ppm). Three-dimensional heteronuclear single-quantum coherence (HSQC) total correlation spectroscopy (TOCSY) spectra, and two-dimensional HSQC

spectra were measured using a Varian Inova 500 MHz spectrometer. HSQC was recorded using spectral widths of 25 kHz in F1 and 5.7 kHz in F2. Two hundred time increments were recorded with 80 transients using a 1-s relaxation delay. The corresponding HSQC-TOCSY parameters were 25 kHz, 5.7 kHz, 220, 400, and 1 s respectively. A spin-lock (MLEV-17) period of 36 ms was used. Both data sets were collected at 50 °C. Spectra were processed using MestReNova software version 6.1.1–6384. Squared sine bell weighting functions were used in both dimensions. ^1H NMR spectra from coarse and milled wood fractions were recorded using a Varian Mercury 300 MHz spectrometer. The samples were dissolved in D_2O (25 mg/mL), and 128 scans were collected at 27 °C with 1 s delay.

X-ray diffraction (XRD) was recorded using a Bruker D8 Advance diffractometer. Dried sawdust and gently homogenized dried sawdust (fraction 1) were deposited in an approximately $10 \times 10 \times 1$ mm layer on the sample holder for the analysis. Raw diffractograms were processed using Microsoft Excel software. Excess noise was removed by plotting the measured data points using a smoothed trendline. Intensity values were normalized based on the raw diffractogram intensities at 5θ .

RESULTS AND DISCUSSION

Structure as a Barrier to Dissolution. In light of earlier work^{20,39–41} and our present data, it seems that coarse particles (around 0.5 mm) of softwood materials are not completely soluble in certain imidazolium-based ILs containing chloride anions. The wood solubility can be enhanced with preparative mechanical degradation, such as ball milling,⁴¹ or catalysts that

Table 1. Data on Fractions^a Collected in This Study, Including Yields, Lignin Contents, and Analyses Performed^{b,c}

solution	Y_{total} (%)	fraction number	Y_{fraction} (%)	lignin content (%)	Y_{lignin} (%)	$Y_{\text{carbohydrate}}$ (%)	analysis
[amim]Cl + 6% 40-mesh TMP spruce, 144 h at 100 °C	86	1	57.4	37.7	75	50	Klason, IR
		2	25.9	12.1	11	32	Klason, IR
		3	0.5	–	–	–	IR
		4	1.8	–	–	–	IR
[amim]Cl + 4% spruce sawdust, 48 h at 90 °C + 96 h at 110 °C	72	1	33.7	52.4	66	22	Klason, IR
		2	31.8	3.8	5	42	Klason, IR
		3	1.3	–	–	–	IR
		4	5.6	–	–	–	IR
[amim]Cl + 4% spruce sawdust, 120 h at 110 °C	93	1	53.2	45.0	90	40	Klason, IR, XRD
		2	33.2	6.2	8	42	Klason, IR
		3	1.3	–	–	–	IR
		4	4.8	–	–	–	IR, ¹ H NMR
[amim]Cl + 10% 28-day-milled TMP spruce, 48 h at 75 °C	86	1	45.7	28.4	45	46	Klason, IR
		2	21.6	36.5	27	19	Klason, IR
		3	2.4	–	–	–	IR
		4	16.3	20.9	12	18	Klason, IR, HSQC, HSQC-TOCSY

^aFraction 5 not included in the table or in yield calculations. ^b Y_{total} , total yield of precipitated material; Y_{fraction} , yield of precipitated fraction from the starting material; lignin content, lignin content of the fraction, including Klason lignin + acid-soluble lignin; Y_{lignin} , yield of lignin in the fraction with respect to the total lignin content in the starting material; $Y_{\text{carbohydrate}}$, yield of carbohydrates in the fraction, including cellulose and hemicelluloses. ^cLignin contents of spruce starting materials were determined experimentally, and those values (28.7% for TMP spruce and 26.6% for spruce sawdust) were used in yield calculations.

cause chemical degradation, such as the presence of acids⁴¹ or oxygen.⁴² Solvation of the wood cell wall seems to start by the dissolution of both the amorphous and crystalline cellulose, as evidenced by compositional and X-ray diffraction data. Other dissolving components are hemicelluloses (about 5% of the wood), according to our earlier data. The compositions of the fractions, as determined by NMR and FT-IR spectroscopies, were found to be mostly 2-*O*- and 3-*O*-acetylated galactoglucomannans. This is in good agreement with the data of Åkerholm and Salmen.³⁴ According to Miyafuji et al.,⁴⁰ the hemicelluloses and lignin also started to dissolve, after the cellulose, at temperatures above 110 °C when 1-ethyl-3-methylimidazolium chloride ([emim]Cl) was used as a solvent. Similarly, we noticed that the temperature should be above 100 °C for wood dissolution, at least for cellulose, but 110 °C was the highest temperature that we used because hydrolysis of cellulose to glucose accelerates rapidly at 120 °C.⁴⁰ An additional reason for the use of lower temperatures was to avoid the decomposition of [amim]Cl, which becomes significant around 150 °C.⁴³

Importantly, spruce TMP fibers and sawdust were found to have different solubilities, despite the relatively large particle sizes, in comparison to finely pulverized samples. Even after 144 h of dissolution at 100 °C, part of the TMP fibers retained a frail recognizable fiber structure, whereas the sawdust had lost most of its fibrous structure, consisting of only small solid fragments. Another indication of differences in dissolution efficiency was that the weight gain values occurring after a benzylation reaction were significantly lower for TMP fibers.³⁶ The solubility seems to depend on the overall form, size, and shape of the particles. By optical microscopy, it was observed that the wood tracheids were maintained notably intact during TMP preparation whereas sawdust preparation destroyed the majority of the fiber structures. The fibrous cell structure in wood can act as a barrier to mass transfer that is crucial for dissolution. Cuissinat et al.⁴⁴ reported the existence of a hardly dissolving band of material in wood and chemical pulp fibers,

other than dissolving pulps, that prevents the homogeneous dissolution of the fibers. This band of recalcitrant material, composed of the primary wall and portions of the secondary wall, allows for the formation of swollen balloon-like structures where the recalcitrant layers are thinnest prior to complete dissolution. Essentially, these layers act as a barrier to dissolution of the more soluble inner cellulosic portion.⁴⁴ Thus, the more preserved fibrous structure of the TMP, compared to the already fragmented cell walls of sawdust, prevents the rapid dissolution of the TMP, in comparison to the sawdust, in which the IL is free to rapidly penetrate all fiber portions in a more homogeneous fashion.

Preparative milling substantially changes the rate and efficiency of wood solvation with an IL, and these can be considered as “true solutions” (homogeneous at least down to the micrometer scale). Increased solubility seems to follow from the disappearance of the fibrous structure and crystallinity and the decreased average molecular weights of the wood polymers. Microscopic examinations revealed that the particles completely lost their crystalline fibrous form that usually responds to polarized light.

Indications of decreased crystallinity during the milling of TMP can be seen in the FT-IR spectra of Figure 2, as evidenced by the disappearance of the band at 1315 cm⁻¹, which is specific to crystalline cellulose.³⁴ In part 1 of this work, we observed a significant decrease in the average molecular weights of samples arising from ball milling.³⁶ Based on the data presented here, we propose that milling especially affects the primary walls and LCC-rich segments of the secondary wall, which might be harder to dissolve because of an extended LCC matrix of effectively infinite molecular weight because of extended bonding between hemicelluloses and lignin. Our results indicate that exposing LCCs to extensive mechanical stresses can cut these structures into small fragments, which results in an increased solubility of the wood in an IL. Cleavage and liberation of the lignin–hemicellulose networks during

milling was evident in the fractionation results, which are discussed later.

Composition of Fractions from Norway Spruce Materials. The first two fractions (fractions 1 and 2) from the IL fractionation of Norway spruce materials, which contained the majority of the mass balance, were precipitated by stepwise acetonitrile addition. These precipitates were extracted with hot water to isolate the water-soluble material into fraction 4. The remaining IL-containing solution was precipitated with water, after near-complete removal of acetonitrile (fraction 3). Even after the water addition, the IL solution could be precipitated further with methanol. After water evaporation, fraction 5 was obtained (Figure 1). The compositions of most of the collected fractions were found to be strongly dependent on the mode of preparation of the starting material (listed in Table 1). FT-IR spectroscopy, Klason analysis, and NMR spectroscopy were selected as the analytical methods in this work because they have been widely used in the area of lignocellulosics. Neutral sugar analysis is traditionally used to characterize the carbohydrate moieties in lignocellulose. However, this method was not practical for us because of the small sample quantities and limited accessibility to chromatographic equipment suitable for accurate neutral sugar analysis. FT-IR spectroscopy was used instead as a fast, nondestructive, and nearly quantitative analytical method, with some earlier published data used as a guide.^{45,46} For our analyses, we used the ratios of the previously identified carbohydrate and lignin bands (using baseline-corrected and normalized spectra) and plotted these ratios against predetermined Klason lignin contents for a variety of samples. A linear fit with an excellent correlation supported our approach. (See the Supporting Information.) Consequently, we used FT-IR spectroscopy as a method to estimate the compositions of the collected fractions.

Fraction 1. Fraction 1 was found to be the most complex in terms of composition, because it contained each of the main wood components in significant amounts, as determined by FT-IR spectroscopy (Figure 2) and Klason lignin content analysis (Table 1). Lignin was found to have been enriched in this fraction when coarse wood materials were used. However, fraction 1 from milled TMP was found to have a lignin content similar to that of the starting material. IR analysis showed that the polysaccharide portion in milled wood fraction 1 consisted of all three wood components, including a significant proportion of cellulose. In the case of coarse materials, this fraction was found to be rich in hemicelluloses and lignin but not cellulose. The intensities of the IR bands related to the skeletal vibrations of the aromatic rings⁴⁵ at 1508 cm^{-1} and the carbonyl bands from acetyl and carboxylic acid groups^{34,46} at 1729, 1603, and 1230 cm^{-1} were seen to increase compared to those for the starting materials. These bands were notably weak in the milled wood sample. A band arising from $-\text{CH}_2-$, mainly attributed to xylan,⁴⁶ was found at 1460 cm^{-1} , and its intensity was seen to behave similarly to the carbonyl band at around 1600 cm^{-1} . Evidence of a prominent cellulose content in the milled sample was obtained from the lack of the aforementioned xylan bands and the band from C2-H in mannose at 811 cm^{-1} ,⁴⁷ in addition to the strong presence of bands at 1370 and 1430 cm^{-1} ascribed to cellulose.⁴⁶ In sawdust fraction 1, the band associated with glucomannan was not particularly prominent, in comparison to the increase over the starting wood in the lignin and xylan carbonyl regions, which suggests that xylan was likely enriched in fraction 1 over

glucomannan (i.e., through partial extraction of glucomannan). The enrichment of the hemicellulose moieties, especially xylan, is consistent with their attachment to lignin through proposed LCC networks.^{23–26,28,29,48}

Fraction 1 represented the fraction present in greatest prominence with yields of 57.4% from TMP fibers, 53.2% from sawdust, and 45.7% from milled TMP powder. The contents of Klason plus acid-soluble lignin in these fractions were 37.7%, 45.0%, and 28.4%, respectively. The higher yields for coarser materials are most likely due to the presence of residual insoluble material and the precipitation of the higher-molecular-weight-range material. XRD analyses of sawdust fraction 1 (Figure 3) revealed a marked decrease in crystallinity,

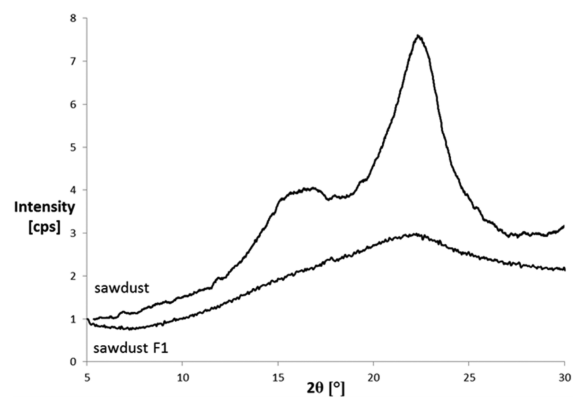


Figure 3. X-ray diffractograms of spruce sawdust starting material and fraction 1 recovered from [amim]Cl.

with the absence of diffraction at 15–17° originating from cellulose I crystalline regions.⁴⁹ This indicates that the IL penetrated the cell walls, altering the crystalline structure of the cellulose but not fully dissolving the whole fiber. An important consequence of this finding is that, if crystalline cellulose is not the key factor preventing complete dissolution, then it is likely that the extended LCC matrix prevents particle reduction upon solvation. This is consistent with literature reports showing that lignin cross-links different polysaccharides into alternating segments,^{25,28} which allows for the formation of large networks. This is particularly relevant for spruce, where almost all isolatable LCCs have been found to be chemically bonded with hemicelluloses but only a minor portion has been found to be chemically bonded with cellulose.²⁸ These results are consistent with the observed preferential extraction of cellulosic material from coarse spruce wood during the IL treatment. This would result in the elimination of crystallinity from the regenerated sample. In contrast, after extended milling, the matrix is fragmented, allowing for different mechanisms of fractionation, namely, more complete solubilization under milder conditions and fractional precipitation, depending on molecular weight.

Fraction 2. The second acetonitrile-precipitated fraction from the coarse spruce sawdust material was found to consist mostly of cellulose with a small amount of lignin. Klason analysis of this fraction gave lignin contents of 6.2% for sawdust and 12.1% for TMP fibers. The total yields for these fractions were 33.2% and 25.9%, respectively. The polysaccharide composition in sawdust fraction 2 was evaluated using FT-IR spectroscopy (Figure 2), and bands arising from hemicelluloses at around 1735 and 810 cm^{-1} were absent, leaving the cellulose bands at 1317 and 1368 cm^{-1} as predominant in the range between 1200 and 1800 cm^{-1} . The band at 670 cm^{-1} indicates

that the polysaccharide moiety in the sawdust fraction 2 was mostly cellulose.⁴⁵ A cellulose-rich composition means that roughly 75% of the 41.7% cellulose content in Norway spruce⁵⁰ actually dissolved in the IL and could be precipitated as a discrete fraction. This would leave fraction 1 as almost completely LCC, only a small portion of which would be soluble and amenable to SEC analysis, after [amim]Cl-mediated benzoylation (see part 1 of this effort³⁶). As mentioned previously, the XRD data in Figure 3 also show a complete reduction in crystallinity, which implies that cellulose was dissolved and extracted during the IL treatment, leaving an amorphous fraction 1 behind, consistent with an LCC matrix.

It is unclear whether the residual lignin in fraction 2 was somehow covalently bonded with the cellulose or free in the solution and precipitated from the IL because of similar factors regarding the molecular weight distribution. However, the yield of lignin (based on the total lignin content in wood) in fraction 2 was similar to values that have been suggested as the amount of lignin bound to cellulose in LCCs.²⁸

Conversely, fraction 2 obtained from milled wood was slightly enriched in lignin. The total yield was 21.6%, with 36.5% of this yield being lignin. The situation with milled wood was fundamentally different from that with sawdust, because all of the fragmented components were found to be solubilized after extensive milling. Overall, the precipitation of cellulose (fraction 2 in the coarse sample and fraction 1 in the milled sample) upon addition of acetonitrile occurred with roughly the same molecular weight distribution for the two different wood samples (see part 1³⁶). However, after precipitation, the molecular weight distribution of lignin seemed to shift from a higher molecular weight (insoluble LCC network) to a lower molecular weight upon preparatory milling and in relation to the cellulose-rich fraction. It seems that fragmentation of the high-molecular-weight LCC network into low-molecular-weight species allows for further fractionation based on molecular weight distribution and not affinity considerations between the IL and the cellulose. This conclusion arises from the data on coarse wood fraction 2 (extractable cellulose).

Fraction 3. A small portion of lignin-rich material did not precipitate upon the addition of acetonitrile for both the coarse and milled materials. Instead, it was precipitated by the addition of a large amount of water, after the acetonitrile had been removed from the IL solution. The yields of this fraction were very low, especially for the coarse materials, varying between 0.5% for TMP fibers and 2.4% for milled TMP. In addition, the average molecular weight of this fraction was found to be lower in the milled sample than in the coarse material.³⁶ One interesting observation was that the IR spectra (Figure 2) from the coarse and milled materials were quite different. Both had intense bands around 1508 and 1263 cm^{-1} related to the aromatic ring and specifically guaiacyl lignin.⁴⁵ Differences were found in the carbonyl band at 1724 cm^{-1} and also in the overlapping bands of aromatic proton and the primary alcohol at 1029 cm^{-1} . Both of these bands, which could be due to the presence of glucuronoxylan, pectin, or fragmented (possibly oxidized) lignin, were much stronger in the sawdust fraction. Cellulose was also present in the coarse, but not the milled, wood fractions.

Fraction 4. Fraction 4 was extracted with water from the acetonitrile-precipitated material (raw fractions 1 and 2; see the Experimental Section) and was regenerated by precipitation of the concentrate from methanol. This allowed the isolation of the water-soluble material over a wide range of molecular

weight distributions, and as such, one would anticipate the hemicellulose-rich material to be located in it because native (acetylated) hemicelluloses are known to be extractable with hot water.^{26,51–54} This was confirmed by the strong IR absorptions observed at 1729, 1230, and 810 cm^{-1} (Figure 2). Chemical shifts of resonances in the HSQC and HSQC-TOCSY NMR spectra due to xylan and glucomannan residues were also apparent. (HSQC and HSQC-TOCSY NMR data are provided in the Supporting Information.) As observed for fraction 2, the nature of this water-soluble fraction was also highly dependent on the mode of preparation and the intensity of milling. The first notable observation is that the yield increased 4-fold after milling. Klason analysis showed that the lignin content in this material increased to 21%, from being hardly detectable in the sawdust fraction (FT-IR and ^1H NMR spectroscopies). (^1H NMR data are also provided in the Supporting Information.) It is likely that these results reflect the fragmentation of the LCC network, allowing for the release of lignin-rich fragments. Lignin itself is not significantly soluble in water at neutral pH if it is in a polysaccharide-free form. Therefore, the presence of lignin connected to hemicelluloses explains why at least 12% of the total lignin (with molecular weights of >1000 Da) was soluble in water. Overall, this is additional confirmation of insoluble LCCs fragmenting and becoming soluble after milling. This is also noticeable when comparing the lignin and polysaccharide contents of fractions 2 and 4 from the milled wood sample. For water-soluble fraction 4, the lignin content was high, but the polysaccharide content was noticeably higher than that in fraction 2. It seems that fragmented LCCs with somewhat higher polysaccharide contents (more specifically, hemicellulose contents) facilitate the solubilization of the lignin in an aqueous environment. In an earlier work, Aimi et al.²⁶ reported the isolation of similar water-soluble fractions in which small lignin fragments were shown to be attached to hemicelluloses.

When fraction 4 from milled TMP was analyzed using the HSQC and HSQC-TOCSY NMR techniques, in addition to typical hemicellulose and lignin resonances, some correlations were found that seemed to correspond to linkages between lignin and xylan. (Resonances assigned from the HSQC spectra and corresponding correlations found from the HSQC-TOCSY spectra can be found in the Supporting Information.) No correlations were observed with glucomannan, but this is hard to verify because $\text{C}\gamma$ in $\beta\text{-O-4}$ structures and C6 in mannose have almost identical carbon chemical shifts and, thus, possible correlations from lignin are overlapped by correlations between mannose resonances. Several resonances could not be identified, but they were likely attached to some other residues. Based on NMR analyses the resonance assigned as the γ -carbon in coniferyl alcohol could also arise from the γ -carbon in a $\beta\text{-O-4}$ -type structure attached to C6 carbon in glucuronic acid by an ester linkage.^{55,59} A few resonances were found that are not found in typical lignin or polysaccharide spectra available in the literature, but they suggested the possible presence of double-bond structures.⁵⁹ This could originate from the allyl group in [amim]Cl, or from some as-yet-unrecognized lignin or polysaccharide decomposition product(s).

The ^1H NMR (Supporting Information) and IR data support the observation that the main component in fraction 4 from sawdust was acetylated glucomannan. Xylan was practically missing from sawdust fraction 4, but it was present in milled wood fraction 4 in significant quantities (according to IR and two-dimensional NMR spectra). This implies that lignin is

frequently linked to at least xylan within the LCC network and that at least some of the galactoglucomannan is not linked to lignin. This is not surprising in view of the literature account of Lundqvist et al.,⁵⁴ who isolated glucomannans by hot-water extraction. Under harsher conditions, fractions having considerably higher molecular weights, around 70000 g/mol, consisting of lignin and xylan were isolated. Milder extraction conditions seemed to afford lower-molecular-weight galactoglucomannan-rich fractions. Similarly, in our experiments, both the lignin and xylan contents simultaneously increased after milling. Again, this suggests that a portion of the galactoglucomannan was free to be extracted with the IL and could be separated from the cellulose by precipitation with a nonsolvent and aqueous extraction. For the milled sample, the lower-molecular-weight LCC fragments precipitated only after cellulose precipitation, and the higher-hemicellulose-content fragments were extractable with water. The LCC fractions 2 and 4 had similar molecular weight distributions, and it is likely that the majority of water-soluble fraction 4 was LCC fragments, originally abundant in unwashed LCC-rich fraction 2.

Fraction 5. Fraction 5 was determined to consist of all of the components that were present in the IL solution after water precipitation and that are insoluble in methanol. According to our IR analyses (see the Supporting Information), this fraction consisted mainly of polysaccharides that were determined to have a molecular weight of 7000 g/mol, probably originating from partial degradation taking place during dissolution or milling. The most significant IR absorptions were at 1315 and 1369 cm^{-1} , which were assigned to cellulose. In addition, small bands at 1732 and 805 cm^{-1} related to glucomannan were also present. Hemicelluloses and lignin seemed to be mostly absent from this fraction. Overall, the yield from the sawdust solution (dissolution conditions of 48 h at 90 °C + 96 h at 110 °C) was 5%, whereas the yield from milled TMP solution is not known (24 h at 80 °C, data not determined). This possibly indicates the retention of residual lower-molecular-weight fractions in the IL even after precipitation with methanol. This is to be anticipated for such fractionation schemes on substrates as complex as wood. These issues represent future challenges for IL recycling and process sustainability, as per our earlier conclusions.⁶⁰

CONCLUSIONS

In part 1 of this series, we documented that the composition of IL-dissolved and reprecipitated wood is dependent on the relative molecular weights of the fractions and not their chemical composition.³⁶ In this work, we further analyzed the fractions for their chemical compositions and found that LCCs are pivotal in determining which components of wood can be fractionated during a mild IL treatment. Surprisingly, it is possible to extract a large proportion of the cellulose from spruce sawdust using this method. The lignin and hemicellulose contents of this extractable material are sufficiently low to allow for bleaching so as to afford the production of a high-purity cellulose pulp. The majority of the lignin in spruce wood is therefore concluded to be bound in a recalcitrant LCC matrix that does not dissolve upon mild treatment with an ionic liquid. Interestingly enough, this matrix does not hinder the extraction of the lower-molecular-weight cellulose. It is only when TMP fibers were used that cellulosic extraction was somewhat hindered. This indicates a strong dependence on particle size. In addition to the ionic-liquid-extractable cellulose, a portion of

the galactoglucomannan is free to be extracted from the recovered fractions using hot water and is not tied up in the LCC network. This water solubility of hemicelluloses also seems to afford some water solubility to lignin LCCs after a harsher treatment (LCC fragmentation due to milling or hot-water extraction). LCC fragments with lower hemicellulose contents seem to remain insoluble in water but are recoverable in the lower-molecular-weight fractions. This water solubility could provide a further handle for fractionation of materials pre- or post-IL treatment. Based on these conclusions, we now aim at designing a fractionation scheme in which the extended LCC network is subjected to a sequence of specific treatments (autohydrolysis,^{56,57} mild acid or mild alkaline treatments⁵⁷), so as to afford increased solubility of the starting wood and potentially a more selective fractionation of individual polysaccharide and nonpolysaccharide components (including potentially soluble LCC). Initial reports combining autohydrolysis, or other pretreatments, with ionic-liquid-mediated fractionation are already appearing that can testify to the validity of our approach.⁵⁸ Overall, intense preparatory milling does not provide a good starting point for component fractionation and is undoubtedly too expensive to compete with chemical pulping. It might, however, afford a method for isolating LCCs.²⁸ From a practical point of view, the extended LCCs network in spruce allow for the rather efficient and chemical-reagent-free separation of cellulose from the intact wood. It remains to be seen if this approach can be extended to increasing particle size and eventually to industrial chips.

ASSOCIATED CONTENT

Supporting Information

¹H NMR spectrum of dried [amim]Cl, FT-IR spectrum of sawdust fraction 5, estimation of lignin contents from FT-IR analysis, ¹H NMR spectrum of sawdust fraction 4, HSQC and HSQC-TOCSY spectra of milled TMP fraction 4 with assignments. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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