

# Fractionation of Lignocellulosic Materials with Ionic Liquids. 1. Effect of Mechanical Treatment

Timo Leskinen,<sup>†</sup> Alistair W. T. King,<sup>†</sup> Ilkka Kilpeläinen,<sup>†</sup> and Dimitris S. Argyropoulos<sup>\*,†,‡</sup>

<sup>†</sup>Department of Chemistry, University of Helsinki, P.O. Box 55, 00014 Helsinki, Finland

<sup>‡</sup>Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27695-8005, United States

 Supporting Information

**ABSTRACT:** The selective precipitation of wood dissolved in 1-allyl-3-methylimidazolium chloride ([amim]Cl) with a nonsolvent is a straightforward method for fractionating lignocellulosic components. In this study we have solvated and precipitated fractions of pulverized Norway spruce (*Picea abies*) and *Eucalyptus grandis* wood. This was achieved by agitating and heating the lignocellulosic materials in [amim]Cl followed by precipitation using nonsolvents, such as acetonitrile (MeCN) and water. Water was also used to extract material, which was determined to be high molecular weight galactoglucomannan. Products were analyzed by benzylation followed by size-exclusion chromatography (SEC) and IR. It was found that the selectivity of precipitation was not significantly dependent upon the chemical composition of the precipitating components. The efficiency of precipitation was found to be dependent upon molecular weight, with the dissolved higher molecular weight and partially soluble wood components precipitating first. Moreover, when coarse sawdust samples were fractionated, the selective dissolution of cellulose from the fiber was observed, which allowed for the regeneration of a fraction of delignified and bleachable cellulose. Additionally, finely milled softwood samples, with demonstrated narrowly distributed low molecular weights, did not efficiently fractionate most likely due to the presence of an extensive lignin–carbohydrate complex (LCC) network.

## INTRODUCTION

Novel techniques for wood fractionation have become a topic of interest after ionic liquids (ILs) were recently found to effectively solvate wood.<sup>1,2</sup> Increased interest from leading groups within the IL community have further established ILs as promising tools for biomass fractionation.<sup>3,4</sup> While fractionation of lignocellulosics could provide commodity and fine chemicals, ILs are also finding application in the treatment of lignocellulosics for the intended production of energy fuels, as described in one detailed investigation by Çetinkol et al.<sup>5</sup> At the moment confidence is high that IL technology will find its way into biorefineries in the future. The academic know-how and the development of sustainable and cost-effective methods are, however, still some distance away, in comparison to existing processes. Undeniably, a key long-term goal is the effective separation of the purified components from wood and grass-based lignocellulosic feedstocks.

On the basis of the superior solvating capabilities of dialkylimidazolium-based ILs, Sun et al. were the first to describe predissolution of wood, followed by regeneration of enriched lignocellulosics by the addition of a nonsolvent.<sup>4</sup> This involved predissolution of yellow pine, as a softwood species, and red oak, as a hardwood species, into ILs such as 1-butyl-3-methylimidazolium chloride ([bmim]Cl) and 1-ethyl-3-methylimidazolium acetate ([emim][OAc]). Precipitation was effectively carried out using acetone/water (1:1 v/v) giving lignin reductions of 26.1% for the softwood and 34.9% for the hardwood after one precipitation cycle. It was observed that higher wood loadings gave lower lignin contents of the initially regenerated materials. A similar result was reported by Lee et al.<sup>6</sup> Lateef et al.<sup>7</sup> have

reported the complete separation of purified cellulose and lignin mixtures by a process of saturation in various ILs and selective precipitation with water and ethanol. This kind of highly selective separation of components from woody material has not yet been achieved, presumably as a result of the highly complex nature of wood; including the rigid crystalline nature of intact cell walls and the additional potential for variable LCCs, which are not consistent between species.

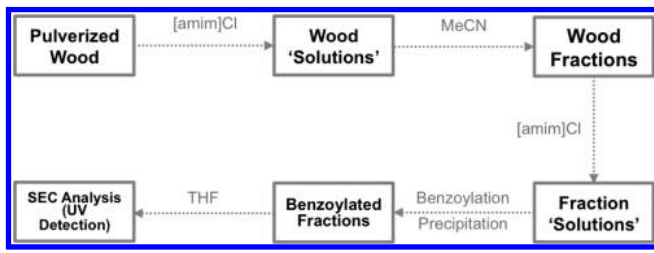
Extraction of certain lignocellulosic components, as opposed to predissolution and selective precipitation, offers an alternative fractionation scheme to that quoted in the literature, i.e., initial reports of fractionation with ionic liquids.<sup>4</sup> Arguably this is the same process as described in these publications, due to a poor definition of what a solution is in these cases and the potentially destructive treatments required for dissolution. Scott et al.<sup>3</sup> used the extraction approach to efficiently separate lignin from cellulose by treatment of bagasse with ionic liquids such as 1-ethyl-3-methylimidazolium mixed alkylbenzenesulfonates ([emim][ABS]). This represents a significant development in the application of ILs for bioprocessing. The applicability of this and other ILs for fractionation of second generation feedstocks however has yet to be proven. However, a recent report by Zoia et al.<sup>8</sup> has highlighted the comparative ease of processing some grass-based feedstocks, such as corn stover, over potential hard and softwood feedstocks. Another recent effective demonstration

**Received:** January 11, 2011

**Accepted:** October 6, 2011

**Revised:** October 3, 2011

**Scheme 1. Schematic Describing the Dissolution, Fractionation, Benzoylation, and SEC Analysis Procedure for the Determination of Molecular Weight Distributions of Fractionated Wood Samples Used in This Study**



of extraction of one lignocellulosic component is in a publication by Abe et al.<sup>9</sup> This publication describes the extraction of high purity cellulose from bran powder at low temperatures with novel ILs such as 1-ethyl-3-methylimidazolium methylphosphonate ( $[\text{emim}][\text{MeHPO}_3]$ ) and 1-ethyl-3-methylimidazolium phosphinate ( $[\text{emim}][\text{H}_2\text{PO}_2]$ ). Bran powder however again represents a first generation feedstock and, as such, is regarded as easier to process than second generation materials like wood.

Ultimately the difficulty in fractionating high molecular weight and preferentially fibrous material from wood is in the solubility of the native material. A recent publication by King et al.<sup>10</sup> demonstrated effectively that the dissolution of Norway spruce into 1-allyl-3-methylimidazolium chloride ( $[\text{amim}]\text{Cl}$ ) under mild dissolution conditions requires pulverization, in the form of planetary ball milling, to achieve material analyzable by solution-state NMR. Even after transparent mixtures are formed it may not be appropriate to refer to these as “wood solutions” but rather suspensions. For concentrations of lignocellulosic solutes high above their critical concentrations, the phase may be homogeneous but better resemble gels. A general term that best describes these states is “solvated wood”, but for ease of use we occasionally refer to them as “wood solutions” on the understanding that they may not be true solutions.

The molecular weight distributions found in intact wood itself have not been extensively studied, using valuable techniques such as size exclusion chromatography (SEC). This has been due to a long-standing inability to effectively solvate wood, while preserving the native molecular structure of the components. However, data is available concerning the molecular weight distributions of wood preparates such as different celluloses,<sup>11,12</sup> pulps,<sup>13–15</sup> hemicelluloses,<sup>16,17</sup> and lignins.<sup>18–20</sup>

The IL  $[\text{amim}]\text{Cl}$  was first described by Wu et al. for the simultaneous dissolution and chemical modification of cellulose.<sup>21</sup> Most of our recent studies concerning wood have involved the use of this IL and, as such, are a suitable basis for studying phenomena during fractionation. One such example details a novel technique for wood solvation, followed by benzoylation and SEC analysis of the UV active product.<sup>8</sup> The authors of the following publication do not yet claim to have developed an efficient or “green” process for the fractionation of wood, using ILs. Rather, by utilizing the capability of these recent analytical and solvation developments, we aim to study in detail the effects of mechanical treatment and species in the fractionation of wood, using  $[\text{amim}]\text{Cl}$  as the solvating media. In spite of the low efficiency of fractionation, the potential of  $[\text{amim}]\text{Cl}$  for the production of viable products will be commented upon.

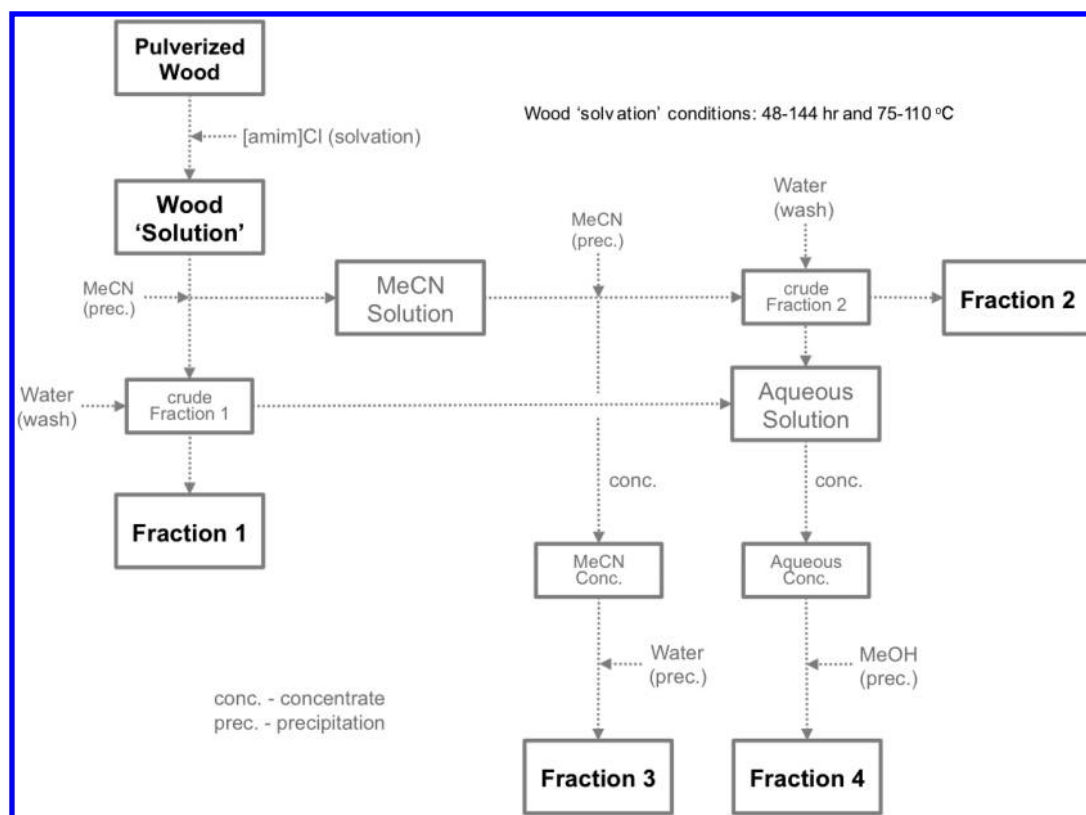
## EXPERIMENTAL DETAILS

Fractionation and analysis of the wood samples by SEC required a procedure involving solvation with  $[\text{amim}]\text{Cl}$ , precipitation with nonsolvent (typically MeCN), and resolution with  $[\text{amim}]\text{Cl}$  followed by benzoylation and SEC analysis (Scheme 1).

**Materials.** MeCN, allyl chloride, ethanol (EtOH), methanol (MeOH), 1-methylimidazole, pyridine (Pyr), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich. Allyl chloride and 1-methylimidazole were distilled prior to use. Unbleached Norway spruce thermomechanical pulp (TMP) was donated from a Swedish mill. Norway spruce sawdust (particle size 0.1–1.0 mm) was prepared from dried heartwood with a belt grinder (grade 60). No contamination of the sawdust from the belt material was found. *Eucalyptus grandis* was supplied by Novozymes, NC. Prior to ball-milling treatments, Norway spruce TMP and corn stover were 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 rotary ball milled in a ceramic plated 5.5 L steel jar with 470 ceramic balls (diameter 0.9 cm) and a rotation speed 60 rpm, for 28 d period. After milling, the fine powder was dried in vacuum oven. The average particle size was determined to be less than 200-mesh ( $75\ \mu\text{m}$ ).

Eucalyptus chips were Soxhlet extracted with acetone for 48 h and then refluxed in 0.075 M NaOH solution (1:50 w/v ratio) for 1 h prior to milling the dried sample. Milling was performed in a Fritsch Pulverette planetary ball-miller, with a 20 mL tungsten carbide grinding bowl and steel balls, at a rotation speed of 420 rpm for 48 h in total. The total milling time was made up of a repetitive milling cycle of 30 min milling time and 20 min brake, to avoid burning the sample. All the wood materials were dried in vacuum oven overnight at 40 °C prior to their use.

**Synthesis of  $[\text{amim}]\text{Cl}$ .** Synthesis of the  $[\text{amim}]\text{Cl}$  was performed according to a method adapted from Wu et al.<sup>21</sup> Allyl chloride (200 mL, 2.51 mol) and 1-methylimidazole (160 mL, 2.01 mol) were added to a flask under nitrogen atmosphere. The mixture was refluxed at 50 °C with stirring under positive pressure of nitrogen for 18 h. The reaction was determined to be complete by  $^1\text{H}$  NMR. The mixture was transferred under nitrogen atmosphere to a rotary evaporator, attached to a high vacuum pump. The excess of allyl chloride was removed at 50 °C. The cloudy crude product was further purified by heating, at 80 °C for 18 h, with activated charcoal (3.0 g) and water (200 mL). The mixture was then filtered through Celite in a grade-3 sinter. Water was removed at 65 °C by rotary evaporation over 18 h, under high vacuum, to yield  $[\text{amim}]\text{Cl}$  as a pale yellow viscous oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  3.97 (3H, s,  $\text{NCH}_3$ ), 4.86 (2H, d,  $J = 6.4$  Hz,  $\text{NCH}_2$ ), 5.33–5.26 (2H, m,  $\text{C}=\text{CH}-\text{C}$ ), 5.86 (1H, ddt,  $J = 16.9$  Hz, 10.3 Hz, 6.5 Hz,  $\text{C}=\text{CH}_2$ ), 7.40 (1H, s,  $\text{C}=\text{CH}$ ), 7.65 (1H, s,  $\text{C}=\text{CH}$ ), 10.39 (1H, s,  $\text{NCHN}$ ). Previously it was determined that  $[\text{amim}]\text{Cl}$  would acidify under vacuum at high temperatures (generally  $>100$  °C) and prolonged periods.<sup>10</sup> The acidity of the material was therefore tested and determined to be neutral, by dissolving  $[\text{amim}]\text{Cl}$  into water (10 mL) and measuring the pH with universal indicator paper. The large batch of  $[\text{amim}]\text{Cl}$  was stored under nitrogen. For the dissolution experiments, 20 g portions were removed from this and thoroughly dried under



**Figure 1.** Illustration of the total fractionation process. Note that the term wood “solution” may not refer to true solutions but rather suspensions.

high vacuum at 65 °C, to give <1% w/w water content prior to use (see Supporting Information for a typical  $^1\text{H}$  NMR spectra).

**Solvation of Wood with [amim]Cl.** Lignocellulosic material (1.0–1.7 g) was quickly added to a flask containing dry [amim]-Cl (10–20 g) under nitrogen atmosphere. The mixture was homogenized with vortex mixer until an even dispersion was obtained. Dissolution was performed in a temperature controlled oil bath using a 3-necked flask under positive pressure of nitrogen. This was equipped with an overhead mechanical stirrer with steel blade. A positive pressure of nitrogen gas was maintained during the whole dissolution period. Solvation conditions and quantities of materials were varied according each experiment performed. Rotary milled Norway spruce powder generally dispersed to give a clear solution in a short period of time. Wiley milled and sawdust materials remained slightly cloudy even after extensive heating at 100 or 110 °C.

**Fractionation of Solvated Wood by Nonsolvent Addition.** Fractions 1–4 were prepared as follows (Figure 1). For fraction 1: When the dissolution period was finished, the temperature of the oil bath was lowered to 70 °C, upon which MeCN (1.7 mL per g of [amim]Cl) was added dropwise, with stirring, to precipitate the first crude fraction. The higher temperature allowed for more rapid homogenization of the sample. After the MeCN addition, the sample was allowed to cool to room temperature (1 h) with stirring. The suspension was allowed to stand for 18 h at room temperature, and the solid precipitate was separated from the solution by centrifugation (5300 rpm, 20 min). The solid precipitate was washed with further MeCN (an additional 1.0 mL per g of [amim]Cl) to remove traces of IL solution. The solid was recovered by centrifugation and the supernatant retained for recombination with the supernatant

from the fraction 1 precipitation. The precipitate was washed with water and centrifuged and the supernatant retained for further use. The solid from this wash was further heated with water for 3 h at 80 °C to remove any potential traces of [amim]Cl and filtered through a grade-3 sinter. The filtrate was retained for preparation of fraction 4, and the filtrand was dried in a vacuum oven for 18 h at 40 °C to give fraction 1. For fraction 2: A second crude fraction was precipitated from the IL-containing supernatant from fraction 1 by adding further MeCN (and additional 2.3 mL per g of [amim]Cl), which included the MeCN that was used to wash the crude fraction 1. The addition was performed dropwise with rapid magnetic stirring. The suspension was allowed to stand overnight in a refrigerator. After centrifugation, the solid precipitate was further washed (with MeCN and water) and separated using the same method for the preparation of fraction 1. The resulting solid was dried in a vacuum oven for 18 h at 40 °C to give fraction 2. For fraction 3: After the precipitation of fractions 1 and 2, the MeCN supernatant of the base solution was evaporated to dryness giving a majority [amim]Cl mixture. The material remaining in IL was precipitated by adding water (3 mL per gram of [amim]Cl) slowly using rapid magnetic stirring. The suspension was allowed to stand overnight in a refrigerator, and the solid was then separated by centrifugation. The solid was further washed with water and dried in a vacuum oven for 18 h at 40 °C to give fraction 3. For fraction 4: The combined clear aqueous filtrates from the purification of fractions 1 and 2 were concentrated down to cloudy solutions with a volume of ~5 mL. MeOH (30 mL) was added forming a fluffy precipitate. This was filtered in a grade-3 sinter and washed with further MeOH. The material was dried in a vacuum oven for 18 h at 40 °C to give fraction 4.

**Derivatization of Lignocellulose Samples by Benzoylation.** The lignocellulose samples were mixed with [amim]Cl (1:20 w/w), and the mixture was homogenized using a vortex mixer. The samples were heated at 80 °C with magnetic stirring for a 20 h period. Regarding samples for which dissolution was incomplete using this procedure, the sample concentration was reduced to (1:40 w/w) and heating was prolonged to 72 h at 100 °C. The mixtures turned clear during the solvation step, and the colors varied from between brown, to amber, to pale yellow, depending on fraction. While the mixtures were still hot, pyridine (8  $\mu$ L per mg of sample) was added to the mixtures and homogenized using a vortex mixer. The dispersion was allowed to cool to room temperature upon which benzoyl chloride was added (7  $\mu$ L per mg of sample). The mixture was vortexed until a homogeneous cloudy paste was formed. Mixing was continued for 2 h at 50 °C with slow magnetic stirring. An EtOH–water mixture (3:1 v/v) was added, and the mixture was vigorously shaken by hand, causing a pale fluffy precipitate to form. The solid was removed by centrifugation (5300 rpm for 10 min) and further triturated with EtOH at room temperature. The solid was again removed by centrifugation and triturated with MeOH for 3 h at room temperature. The methanolic suspension was filtered through a grade-3 sinter, and the resulting solid was dried in a vacuum for over 18 h at 40 °C to give pale white to brown benzoylated products. The masses of the recovered products were weighted after drying, and weight percentage gains (WPGs) were calculated to vary from between 31% and 141%, depending on the sample.

**Molecular Weight Distribution by SEC.** The benzoylated samples were dissolved into THF (1 mg per mL). All samples from milled wood dissolved almost completely. From materials derived from sawdust and wiley milled powders, the starting materials and fraction 1 samples were either insoluble or only partially soluble, even after dissolution was performed using the harsher conditions. The solutions were filtered through a 0.45  $\mu$ m PTFE filter, and 200  $\mu$ L volume samples were injected onto the columns.

The SEC system consisted of a HP G1312A pump connected to Waters HR5E and HR1 columns with a Waters 484 UV-absorbance detector. Eluents were detected at 208 nm. THF was used as eluent with a 0.7 mL min<sup>-1</sup> flow rate. Before measurements, the system was calibrated using narrow polystyrene standards ranging from 944 000 to 820 g mol<sup>-1</sup> and vanillin (152 g mol<sup>-1</sup>). The system was controlled with Millennium 32 SEC software. Processing of the data was carried out using the same software.

## RESULTS AND DISCUSSION

**Solvation Conditions.** For the separation of wood components via dissolution and precipitation, it is desirable that wood is completely dissolved. Unfortunately, full solubility is not achievable under mild conditions, at least with wood chips or corresponding coarse materials in [amim]Cl or [bmim]Cl.<sup>4,10</sup> The same observation was made during this study. The problem with incomplete solubility can be overcome by preparative milling of the fibrous wood material. King et al.<sup>10</sup> demonstrated that Norway spruce sawdust can be fully dissolved into [amim]Cl after 48 h ball-milling, under mild and acid-free dissolution conditions. In this previous report, all hydroxyl groups in wood can be phosphitylated after extensive milling and the <sup>31</sup>P resonances observed by 1D NMR, against an internal standard. Aside from being a useful analytical procedure for quantifying

different hydroxyl groups in wood, this publication demonstrated that wood, under mild dissolution conditions, is not fully soluble in ILs such as [amim]Cl. It is debatable whether [emim][OAc] has any additional solvating power that allows for increased solvation capabilities, but is unlikely to fully dissolve wood due to the complex and crystalline nature of wood. To account for this positive effect arising from milling, it has been demonstrated how milling breaks bonds between lignin monomers.<sup>10,18</sup> Cellulose and other polysaccharides are also degraded under mechanical stress.<sup>22–24</sup> Reduction in molecular weights resulting from breakage of the native structure seems to be the key to complete dissolution of wood in IL. This can be achievable from either mechanical treatment or chemical treatment. In effect, the result is a reduction in the “effective” molecular weight of the complex, covalently linked and entangled network of lower molecular weight biopolymers. The result is solubilization in the ionic liquid media.

In this study, we compared the efficiency of fractionation of “solutions” made by mixing coarse Norway spruce sawdust, wiley-milled 40 mesh Norway spruce TMP, and the same spruce TMP after 28 d rotary ball-milling into [amim]Cl. Although this study was mainly concentrated on spruce softwood, we also include ball-milled eucalyptus as an example of a hardwood. We chose to include preparative milling for our fractionation studies. The main reason for this is that we required components to be fully dissolved. This would allow us to test fractionation using conditions as close to a solution as possible. This would be in accordance with current fractionation concepts or understanding quoting total dissolution and selective precipitation of wood fractions.<sup>4</sup> This could potentially allow us to determine appropriate solvent conditions and selectivity for fractionating one component, on the basis of chemical composition. As expected, the complete dissolution of coarser materials was not possible, without using much higher temperatures that might cause degradation. This partial solvation also had an effect on the efficiency of chemical modification of certain fractions and resulted in only partial solubility of some of the benzoylated materials, prior to SEC analysis. With the finely milled samples, we can visualize the effect of milling on the whole molecular weight distribution of the fractions, as opposed to only the soluble portion of the materials prepared from the coarse samples, although higher molecular weight distributions for the coarser materials are still apparent.

Solvation with [amim]Cl was performed using a system with a temperature controlled oil bath at temperatures between 75 and 110 °C, mechanical stirrer, and nitrogen atmosphere. This arrangement guaranteed a stable temperature, an effective thorough mixing, and an inert atmosphere as aerial oxidation has been shown to cause depolymerization of wood components in 1-ethyl-3-methylimidazolium chloride ([emim]Cl).<sup>25</sup> For the milled wood samples, transparent “solutions” were achieved in 48 h at only 75 °C. The sawdust and coarse TMP fibers however required dissolution times between 120 and 144 h at 100–110 °C. Although these mixtures seemed transparent, optical microscopy showed silhouetted fragments remaining as opposed to completely clear solutions for the finer pulverized materials.

**Fractionation of Wood Solutions and Nonsolvent Choice.** In preliminary experiments, several traditional molecular solvents were tested on finely pulverized milled wood samples. Polar protic solvents such as water, short-chain alcohols, and acetic acid were able to precipitate dissolved material efficiently. In all cases, gel-like materials or emulsions were formed which, for fractionation purposes, are not favorable. The relatively polar

**Table 1.** Total Yield ( $Y_{TOT}$ ), Yield of Fraction ( $Y_{FRAC}$ ), Weight Percent Gain after Benzoylation (WPG), Number Average Molecular Weight ( $M_N$ ), Weight Average Molecular Weight ( $M_W$ ), and Polydispersity (PD) for Collected Fractions

	$Y_{TOT}$ (%)	fraction number	$Y_{FRAC}$ (%)	WPG (%)	$M_N$ ( $\times 10^3$ g mol $^{-1}$ )	$M_W$ ( $\times 10^3$ g mol $^{-1}$ )	PD	lignin content (%)
[amim]Cl, 6% 40-Mesh TMP Spruce, 144 h 100 °C								
		1	57.4	86	2.3 <sup>a</sup>	7 <sup>a</sup>	2.9	
	86	2	25.9	120	7.5	71	9.5	12.1
		3	0.5		2.5	12	4.7	
		4	1.8					
[amim]Cl, 4% Spruce Sawdust, 120 h 110 °C								
		1	53.2	104	4.8 <sup>a</sup>	24 <sup>a</sup>	4.9	45.0
	93	2	33.2	141	11.5	48	4.1	6.2
		3	1.3		2.4	8	3.3	
		4	4.8	70	9.6	30	3.1	
[amim]Cl, 10% 28 d Rotary Milled, TMP Spruce, 48 h 75 °C								
		1	45.7	104	9.2	40	4.4	
	86	2	21.6	120	6.6	22	3.3	36.5
		3	2.4		1.6	4	2.5	
		4	16.3	85	8.8	26	2.9	
[amim]Cl, 10% 48 h Planetary Milled Eucalyptus, 48 h 75 °C								
		1	42.1	99	11.4	95	8.3	
	59	2	12.7	101	6.2	30	4.9	
		3	4.2	53	1.4	9	6.3	
Starting Materials								
				103	1.9 <sup>a</sup>	5 <sup>a</sup>	2.9	28.7
				133	5.5 <sup>a</sup>	34 <sup>a</sup>	6.1	26.6
				98	5	31	6.2	26.1
Reference Materials								
					10.7	1 727	161	0
					12.5	1 212	96.6	0

<sup>a</sup>The sample was not totally soluble in THF,  $Y_{total}$  = total yields of precipitated material,  $Y_{fraction}$  = yield of single fraction from starting material.

chlorinated solvent, chloroform, had the tendency to mix well with [amim]Cl. The efficiency of precipitation with this solvent was quite good, but due to the difficulties in treating waste streams containing chlorinated solvents, this choice was avoided. Strongly polar aprotic solvents DMSO, DMF, and MeCN were found to be very suitable for fractionation, to yield solid to semisolid material. From these three candidates, MeCN was chosen for two good reasons. The first is that it is volatile, and can be easily removed from the IL for change of nonsolvent or recycling. The second reason is that out of the solvents screened, MeCN by far afforded the most optically bright fractions. This would allow for easier bleaching to low-grade pulp if required.

The complete fractionation scheme is described in detail in the Experimental Section subheading titled Fractionation of Solvated Wood by Nonsolvent Addition and is illustrated in Figure 1. The mass balances, molecular weights, and klason lignin content analyses are presented in Table 1. Only a small portion of data detailing the chemical composition of the fractionations will be presented here as further publications will talk about the chemical composition in detail, while the present publication is dedicated toward studying mainly the effect of pulverization on fractionation. In certain instances, experimental data detailing the chemical composition of fractions (e.g., from Klason analysis or IR) is presented to support certain conclusions.

#### Effect of Molecular Weight versus Chemical Composition.

By studying the mass recoveries and the yields of the fractions, it is obvious that more lignocellulosic material is retained in the IL from "solutions" prepared from the finely pulverized samples. The coarse samples were almost completely precipitated with the two portions of MeCN. By washing the crude fractions 1 and 2 with water, as a polar protic solvent, a further small quantity of material is extracted. This extracted material was then found to be insoluble in MeOH. Also, as a polar protic solvent, MeOH was found to precipitate a small amount of additional material from the IL solutions, after MeCN removal. After this, the mass balances indicate that residual material still remains in the IL solution, probably of low molecular weight oligomeric materials.

The molecular weight distributions for the fine eucalyptus fractions are illustrated in Figure 2. The molecular weight distributions for the coarse spruce sawdust fractions 1–4 and finely pulverized spruce TMP fractions 1–4 are visualized in Figure 3. For the finely pulverized eucalyptus sample, there is a visible decrease in molecular weight from fraction 1 to fraction 3 (Figure 2). For the finely pulverized spruce sample, there is also a visible decrease in molecular weight upon going from fraction 1 to fraction 3. Although sawdust fraction 1 demonstrates a slightly lower molecular weight in comparison to fraction 2 in Figure 3, we can assume it actually has a much

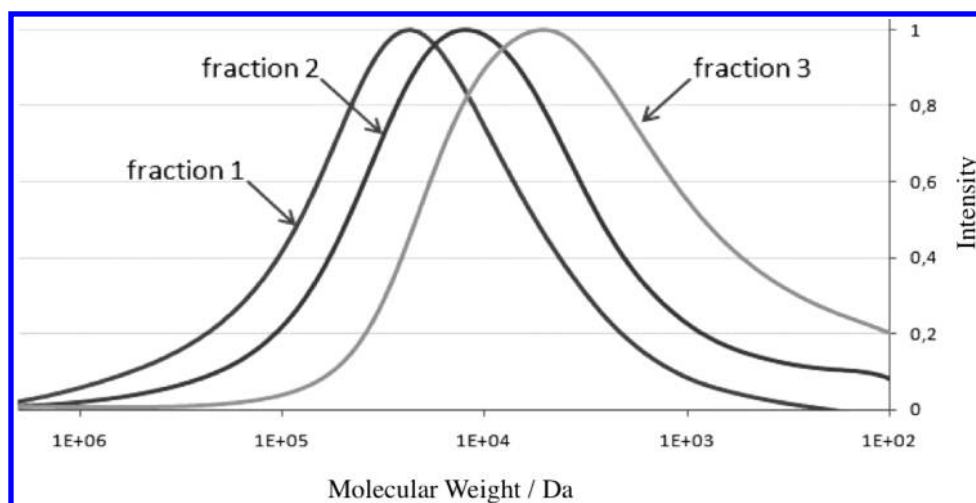


Figure 2. SEC-chromatograms of separated fractions from solution of eucalyptus.  $M_W$ -values are presented against normalized intensities.

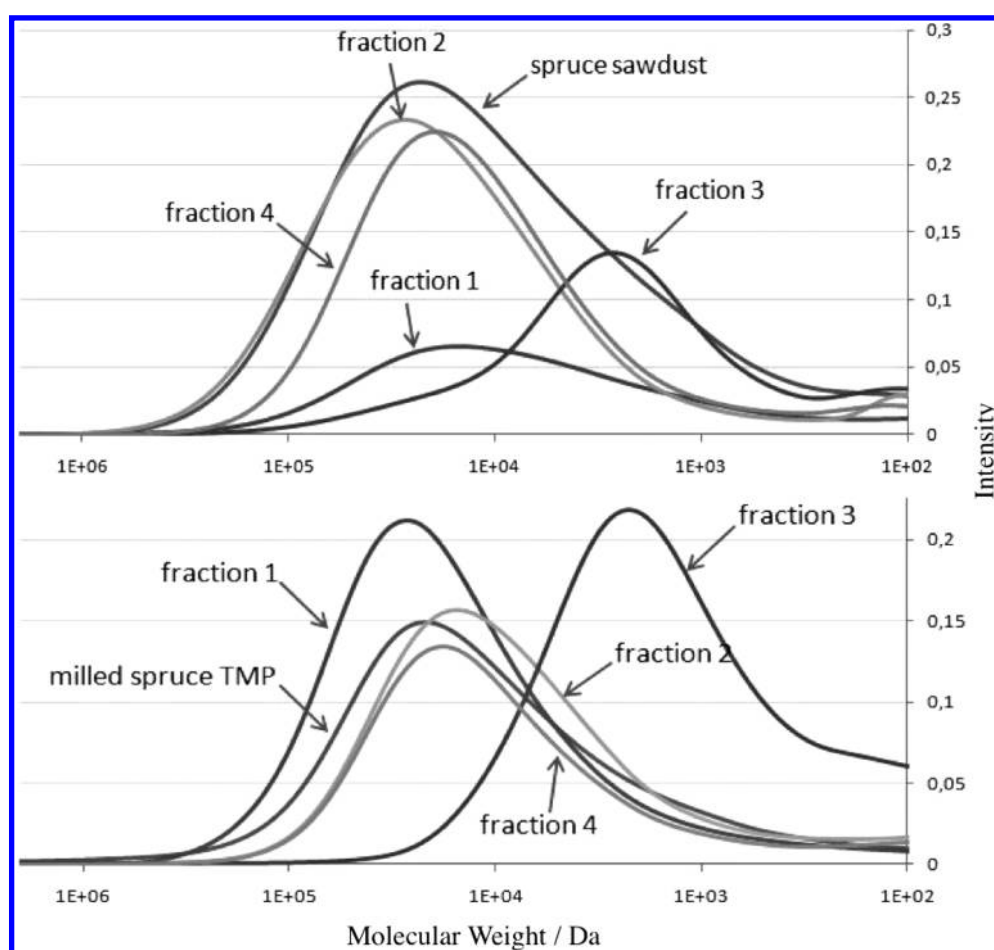
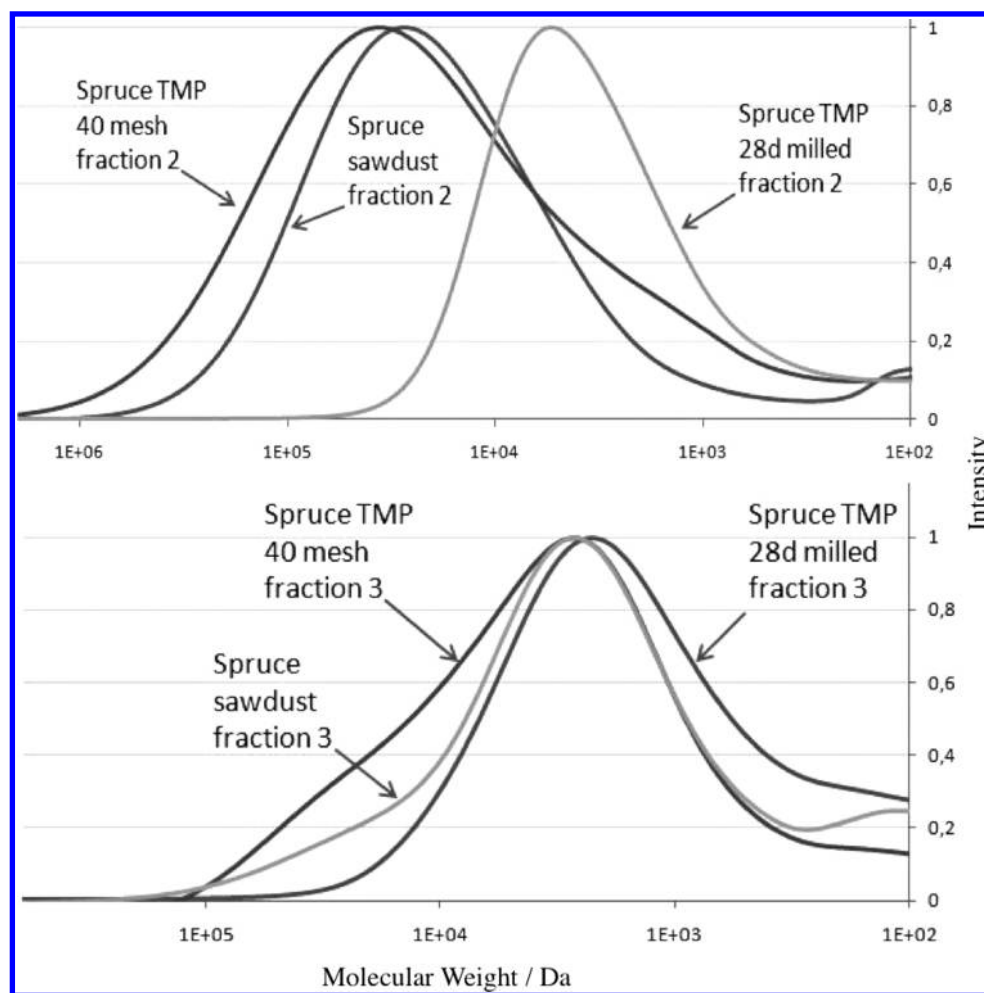


Figure 3. SEC-chromatograms of separated fractions from solutions of spruce sawdust (above) and 28 d milled spruce TMP (below) including the starting materials.  $M_W$ -values are presented against original intensities.

higher molecular weight due to the amount of insoluble material remaining after dissolution into THF. This decrease in molecular weight with fractionation is further illustrated by comparing the molecular weight distributions of fractions 2 and 3 from all the spruce samples (Figure 4). From a comparison of the

$M_W$  values of the fraction 2 samples, there is a clear decrease in molecular weight on going from the  $71\,000\text{ g mol}^{-1}$  of 40-mesh TMP to the sawdust with  $48\,000\text{ g mol}^{-1}$  and then to the finely milled sample having only  $22\,000\text{ g mol}^{-1}$ . The fraction 3 samples however maintain similar molecular weight



**Figure 4.** Comparison of corresponding fractions 2 (above) and fractions 3 (below) from used starting materials.  $M_W$ -values are presented against normalized intensities.

distributions between the coarse and fine samples, with only a slight increase in  $M_W$  values on going to the coarse fractions. Therefore, for all samples it can be said that precipitation at least is highly dependent on the molecular weight of the materials. This is especially true in the case of the coarse samples that did not even fully dissolve prior to fractionation. This phenomena potentially prevents any selectivity in fractionation that may be afforded by actual dissolution into and precipitation from [amim]Cl. This does not necessarily mean however that selective fractionation is not occurring but still allows for the possibility that there is some selective solubilization of different wood biopolymers from the native sample. If one can extract components such as hemicelluloses using hot water, then it may be fair to say that ILs can selectively extract the same or other components not extensively and covalently bound to the extended wood matrix.

To shed more light on this issue, klason lignin content values are presented for fractions 2 of the spruce samples and for fraction 1 of the 4% spruce sawdust sample. From analysis of the fraction 2 samples it can clearly be seen that the lignin content of fraction 2 increases upon increasing the degree of pulverization. For the sawdust sample, this is a strong indication that as the fibrous sawdust sample is dissolving, the lignin is remaining relatively untouched and that it is some polysaccharide component

that is soluble in the IL. This is contrary to what Sun et al.<sup>4</sup> have demonstrated using [emim][OAc] as IL. They reported a decrease in lignin content, presumably by extraction, after cooking both pine and oak fibrous material and regenerating the cellulosic equivalent of our fraction 1. This may indicate differences in the mode of dissolution between the two ILs [amim]Cl and [emim][OAc] or it may also result from differences in the structure of the wood between the species. IR analysis of fraction 2 (see Supporting Information) confirmed the material to be a majority of cellulose, due to the absence of any IR resonances attributable to galactoglucomannan, as the major hemicellulose in softwoods. Fraction 1 however was shown to contain galactoglucomannan and 45% of lignin. Fraction 2 for the finely milled spruce sample also shows a moderate enrichment of the lignin, not depletion as with the coarse samples.

These results gives us enough information to draw some important conclusions about fractionation of wood using [amim]Cl. The first conclusion is that lignin in native wood is not particularly soluble in the IL and cellulose seems to be the more extractable or soluble biopolymer, probably mostly from its amorphous regions. The second conclusion is that molecular weight plays a much larger part in the efficiency of fractionation than the actual chemical composition. As opposed to the efficient fractionation of lignin from cellulose, as described by Lateef et al.,<sup>7</sup>

we did not observe any significant efficiency in fractionation, even when the sample was pulverized to the low molecular weights that you might find in Lateef's study. Fraction 2, from the finely milled sample, has an  $M_W$  value of  $30\,000\text{ g mol}^{-1}$  which would correspond to a DP of  $\sim 185$  if it were cellulose. This is a lower DP than some preparative cellulose grades. The fact that this fraction retains and slightly enriches in lignin, in comparison to the Lateef fractionation, can be explained by the presence of covalent linkages that exist between some polysaccharide component and the lignin. This is not surprising as Lawoko et al.<sup>26</sup> have shown that hemicelluloses bond covalently with lignin in spruce and that these LCCs are not formed during the mechanical treatment step.

The water solubility of fraction 4 could be argued to be due to the presence of acetylated hemicelluloses. IR analysis (see Supporting Information) confirms the presence of galactoglucomannans. Surprisingly, they have a considerably higher molecular weight, in comparison to hot water extracted galactoglucomannan, as reported by Lundqvist et al.<sup>16</sup> The  $M_w$  of our sample was  $30\,000\text{ g mol}^{-1}$  while the water-soluble fraction from Lundqvist was under  $5000\text{ g mol}^{-1}$ . Jacobs et al.<sup>17</sup> have used KOH to extract spruce hemicelluloses that have  $M_w$  around  $20\,000\text{ g mol}^{-1}$ . In light of these results the high  $M_w$  of the water-soluble fraction does not seem surprising. The fractions of Jakobs however had two times higher  $M_N$  which can be an indication of slight degradation during our IL treatments.

**State of the Art Application of Ionic Liquids for Fractionation.** In relation to developing a suitable fractionation process, it can be observed that for the 4% spruce sawdust sample, we can isolate fraction 2 as a material, which is essentially delignified and almost completely composed of cellulose. This can be regarded as a bleachable grade pulp and after bleaching would result in high purity cellulose akin to dissolving pulp. The  $M_W$  values are however rather low in comparison to existing fractionation technologies and materials. In comparison to softwood kraft and sulfite pulps<sup>13,14</sup> using other SEC methods, the  $M_W$  values of our fractions are only about  $1/10$  of the corresponding values for the pulps. Using our own method,<sup>8</sup> we analyzed bleached softwood pulp and cotton for comparison purposes, and we obtained values of  $\sim 20$ – $30$  times the MW for fraction 2 from 4% spruce in [amim]Cl (Table 1). Needless to say, the fractionated material retained no fibrous properties and could not be termed "pulp". The material could however be chemically modified further for more advanced applications. In regard to generating a fibrous "pulp", as dissolution of the coarse sawdust continues to completion, this is likely accompanied by initial degradation of the amorphous cellulose and ultimate solubilization of the material, destroying the fibrous qualities of any regenerated materials. Dissolution, by definition, destroys the fibrous ultrastructure of wood. This fibrous ultrastructure has been designed by nature and assembled by natural processes, not by man-made self-assembly processes.

An alternative process may come in the form of a pretreatment of wood for further processing. High molecular weight hemicelluloses can be commonly extracted with a water treatment. This partial dissolution of intact fibers with IL, accompanied by gradual degradation of the material, may allow for increased yields and molecular weights of hemicelluloses. Again, if this process is to be made viable, it is likely that yields of extraction will likely correlate with a reduction in the fibrous properties of the recovered fractions.

## CONCLUSIONS

A comparison of the fractionation of coarse and finely pulverized wood from [amim]Cl identified that fractionation mainly is dependent on molecular weight and not chemical composition. This was observed for a softwood (Norway spruce) and a hardwood (Eucalyptus) species. When finely milled wood is completely dissolved in [amim]Cl, most of its components can be precipitated in order of decreasing molecular weight using MeCN and MeOH as nonsolvents. Contrary to reports of highly efficient fractionation of cellulose doped with high purity lignin,<sup>7</sup> it was only possible to fractionate our finely pulverized and dissolved spruce wood with marginal selectivity. It is likely that the presence of extensive LCCs, especially within the softwood material, is preventing the separation of lignin and polysaccharides. This suggests the need for a pretreatment of wood so as to break LCCs in order to increase the selectivity of fractionation. In the fractionation of sawdust, it was determined that cellulose preferentially dissolves in the initial dissolution step and that lignin is relatively resistant to being removed from the fiber. After precipitation of the residual insoluble material it was possible to precipitate the dissolved cellulose in bleachable grade and relatively high purity. This result, using [amim]Cl, is contrary to previous publications using [emim][OAc] and suggests either a different mode of dissolution of wood between the two classes of IL or between different species. Additionally, extraction of the residual fibrous and the precipitated materials after the fractionation of sawdust afforded acetylated galactoglucomannan with relatively high molecular weight.

## ASSOCIATED CONTENT

**S Supporting Information.** Characterization of the pure ionic liquid; ATR-IR figures for spruce sawdust and spruce sawdust fractions 1, 2, and 4; references describing the IR characterization of lignocellulosics. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mails: [dimitris.argyropoulos@ncsu.edu](mailto:dimitris.argyropoulos@ncsu.edu), [dsargyro@ncsu.edu](mailto:dsargyro@ncsu.edu).

## ACKNOWLEDGMENT

We wish to thank the Finnish Funding Agency for Technology and Innovation (TEKES) for making this project possible.

## REFERENCES

- (1) Fort, D. A.; Remsing, R. C.; Swatloski, R. P.; Moyna, P.; Moyna, G.; Rogers, R. D. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-*n*-butyl-3-methylimidazolium chloride. *Green Chem.* **2007**, *9*, 63–69.
- (2) Kilpeläinen, I.; Xie, H.; King, A. W. T.; Granström, M.; Heikkinen, S.; Argyropoulos, D. S. Dissolution of wood in ionic liquids. *J. Agric. Food Chem.* **2007**, *55*, 9142–9148.
- (3) Tan, S. S. Y.; MacFarlane, D. R.; Upfal, J.; Edye, L. A.; Doherty, W. O. S.; Patti, A. F.; Pringle, J. M.; Scott, J. L. Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chem.* **2009**, *11*, 339–345.
- (4) Sun, N.; Rahman, M.; Qin, Y.; Maxim, M. L.; Rodríguez, H.; Rogers, R. D. Complete dissolution and partial delignification of wood in



the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chem.* **2009**, *11*, 646–655.

(5) Çetinkol, Ö. P.; Dibble, D. C.; Cheng, G.; Kent, M. S.; Knierim, B.; Auer, M.; Wemmer, D. E.; Pelton, J. G.; Melnichenko, Y. B.; Ralph, J.; Simmons, B. A.; Holmes, B. M. Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* **2010**, *1*, 33–46.

(6) Lee, S. H.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnol. Bioeng.* **2009**, *102*, 1368–1376.

(7) Lateef, H.; Grimes, S.; Kewcharoenwong, P.; Feinberg, B. J. Separation and recovery of cellulose and lignin using ionic liquids: a process for recovery from paper-based waste. *J. Chem. Technol. Biotechnol.* **2009**, *84*, 1818–1827.

(8) Zoia, L.; King, A. W. T.; Argyropoulos, D. S. Molecular weight distributions and linkages in lignocellulosic materials derivatized from ionic liquid media. *J. Agric. Food Chem.* **2011**, *59*, 829–838.

(9) Abe, M.; Fukaya, Y.; Ohno, H. Extraction of polysaccharides from bran with phosphonate or phosphinate-derived ionic liquids under short mixing time and low temperature. *Green Chem.* **2010**, *12*, 1274–1280.

(10) King, A. W. T.; Zoia, L.; Filpponen, I.; Olszewska, A.; Xie, H.; Kilpeläinen, I.; Argyropoulos, D. S. In situ determination of lignin phenolics and wood solubility in imidazolium chlorides using  $^{31}\text{P}$  NMR. *J. Agric. Food Chem.* **2009**, *57*, 8236–8243.

(11) Eremeeva, T. Size-exclusion chromatography of enzymatically treated cellulose and related polysaccharides: a review. *J. Biochem. Biophys. Methods* **2003**, *56*, 253–264.

(12) Strlič, M.; Kolar, J. Size exclusion chromatography of cellulose in LiCl/N,N-dimethylacetamide. *J. Biochem. Biophys. Methods* **2003**, *56*, 265–279.

(13) Yanagisawa, M.; Shibata, I.; Isogai, A. SEC-MALLS analysis of softwood kraft pulp using LiCl/1,3-dimethyl-2-imidazolidinone as an eluent. *Cellulose* **2005**, *12*, 151–158.

(14) Schult, T.; Hjerde, T.; Optun, O. I.; Kleppe, P. J.; Moe, S. Characterization of cellulose by SEC-MALLS. *Cellulose* **2002**, *9*, 149–158.

(15) Sjöholm, E.; Gustafsson, K.; Berthold, F.; Colmsjö, A. Influence of the carbohydrate composition on the molecular weight distribution of kraft pulps. *Carbohydr. Polym.* **2000**, *41*, 1–7.

(16) Lundqvist, J.; Telemann, A.; Junell, L.; Zacchi, G.; Dahlman, O.; Tjerneld, F.; Stålbrand, H. Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydr. Polym.* **2002**, *48*, 29–39.

(17) Jacobs, A.; Dahlman, O. Characterization of the molar masses of hemicelluloses from wood and pulps employing size exclusion chromatography and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Biomacromolecules* **2001**, *2*, 894–905.

(18) Guerra, A.; Filpponen, I.; Lucia, L. A.; Saquing, C.; Baumberger, S.; Argyropoulos, D. S. Towards a better understanding of the lignin isolation process from wood. *J. Agric. Food Chem.* **2006**, *54*, 5939–5947.

(19) Guerra, A.; Gaspar, A. R.; Contreras, S.; Lucia, L. A.; Crestini, C.; Argyropoulos, D. S. On the propensity of lignin to associate: A size exclusion chromatography study with lignin derivatives isolated from different plant species. *Phytochemistry* **2007**, *68*, 2570–2583.

(20) Sjöholm, E.; Gustafsson, K.; Colmsjö, A. Size exclusion chromatography of lignins using lithium chloride/N,N-dimethylacetamide as mobile phase. II. Dissolved and residual pine kraft lignins. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 2837–2854.

(21) Wu, J.; Zhang, J.; Zhang, H.; He, J.; Ren, Q.; Guo, M. Homogeneous acetylation of cellulose in a new ionic liquid. *Biomacromolecules* **2004**, *5*, 266–268.

(22) Ott, R. L. Mechanism of the mechanical degradation of cellulose. *J. Polym. Sci., Part A: Gen. Pap.* **1964**, *2*, 973–982.

(23) Kuzuya, M.; Yamauchi, Y.; Kondo, S. Mechanolysis of glucose-based polysaccharides as studied by electron spin resonance. *J. Phys. Chem. B* **1999**, *103*, 8051–8059.

(24) Iversen, T. Lignin-carbohydrate bonds in a lignin-carbohydrate complex isolated from spruce. *Wood Sci. Technol.* **1985**, *19*, 243–251.

(25) Nakamura, A.; Miyafuji, H.; Saka, S. Influence of reaction atmosphere on the liquefaction and depolymerization of wood in an ionic liquid, 1-ethyl-3-methylimidazolium chloride. *J. Wood Sci.* **2010**, *56*, 256–261.

(26) Lawoko, M.; Henriksson, G.; Gellerstedt, G. Characterisation of lignin-carbohydrate complexes (LCCs) of spruce wood (*Picea abies* L.) isolated with two methods. *Holzforschung* **2006**, *60*, 156–161.