

## Chapter 6 Fractionation of lignocellulosic materials with ionic liquids

Timo Leskinen<sup>a</sup>, Alistair W. T. King<sup>c</sup>, Dimitris S. Argyropoulos<sup>\*a,b</sup>

<sup>a</sup> Departments of Forest Biomaterials and Chemistry, North Carolina State University, Raleigh, USA, 27695-8005,

<sup>b</sup> Center of Excellence for Advanced Materials Research (CEAMR), King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia

<sup>c</sup> Department of Chemistry, University of Helsinki, Helsinki, Finland, FI-00014,

\*Corresponding authors: [dsargyro@ncsu.edu](mailto:dsargyro@ncsu.edu)

### Abstract

Ionic liquids (ILs) have been recognized as a promising way to fractionate lignocellulosic biomass. During recent years, a number of publications have introduced a variety of technical developments and solvent systems based on several types of ILs to fractionate lignocellulose into individual polymeric components, after full or partial dissolution. In this chapter we briefly review the latest developments and knowledge in this field of study and introduce an alternative fractionation method based on the controlled regeneration of components from 1-allyl-3-methyl-imidazolium chloride ([amim]Cl). Norway spruce (*Picea abies*) and *Eucalyptus grandis* woods were dissolved in their fibrous state or by utilizing ball milling to improve solubility. The resulting wood solutions were precipitated gradually resulting into fractions by addition of non-solvents, such as acetonitrile and water. Further water extraction of the crude fractions resulted in better separations. By analyzing molecular weight distributions of the fractions, together with their chemical composition, we have obtained fundamental information concerning the mechanisms of wood fractionation with ILs. Fractionation efficiency is found to be highly dependent on the modification of the wood cell wall ultrastructure and the degree of

reduction of the molecular weights of the main components, arising from mechanical degradation. Isolation of cellulose enriched fractions was achieved with Spruce sawdust and ball milled Eucalyptus, evidently following from distinct dissolution mechanisms.

Keywords: Wood, cellulose, lignin, LCC, ionic liquids, 1-allyl-3-methylimidazolium chloride, fractionation, extraction, separation, molecular weight, pulp, biofuels

## 6.1 Introduction

In a relatively short time, the research area of ionic liquid-mediated fractionation and pretreatment of wood has emerged from the interest of a small group of scientists into a noteworthy and diverse field of study. Global interest in lignocellulosic biomass is experiencing a renaissance, not only because of the growing financial potential in lignocellulose-based liquid biofuels, but also because it represents a source of bio-based materials and chemicals. Ionic liquids (ILs) have been recognized to have potential in many applications that can be categorized under the advanced utilization of grassy and woody biomass. The ability to dissolve various biopolymers and a general status as a green alternative to organic solvents makes IL platform technologies attractive to industry. This is mostly in areas pertaining to manufacturing of novel polymeric materials, by derivatization or blending of cellulose, or in biomass pretreatments, including structural or compositional alteration of plant cell walls and acid catalyzed hydrolysis of plant polysaccharides, for the purposes of biofuel production[1-3]. Aside from the use of ILs as a media for modification, fractionation of lignocellulosic biomass can be integrated into a variety of applications as it can also be used as a method to obtain purified or specified polymeric raw materials, for further use[4]. IL-mediated fractionation is suitable for the general concept of a biorefinery, serving the demand of component separation for subsequent multiple product streams. Ideally this method should be tunable. However, the selectivity of fractionation

of native woods using ILs is still poorly developed or understood, from a mechanistic point of view.

Understanding the fundamentals of the separation of polymeric cell wall components has improved after initial publications concerning cellulose and whole wood dissolution into ILs[5-7]. Ideally, there are two ways to fractionate lignocellulose: (1) complete pre-dissolution of biomass followed by selective precipitation of the sought components as purified fractions, by addition of a non-solvent, or (2) selective extraction of components from the biomass. The first efforts to isolate purified fractions using ILs can be roughly categorized under either of the aforementioned approaches[6,8-10]. However, the complex recalcitrant structure of wood greatly hinders a complete dissolution and efficient fractionation. During the last few years, a variety of new methods have emerged resulting in enhanced fractionation. In addition to introducing our work on wood fractionation, in this effort we will also present a brief overview of the latest technical advances in the IL-based fractionation systems and of our findings related to the mechanisms controlling the dissolution and fractionation of the complex materials of the wood cell wall.

Our work concerning wood fractionation has focused on dissolution of Norway spruce (softwood) and *Eucalyptus grandis* (hardwood) woods, as completely as possible under mild conditions, followed by a stepwise regeneration of wood components, with the addition of non-solvents. From initial screening, non-solvents were chosen in an attempt to enhance the selectivity of component precipitation. In this selection we considered the optical brightness of the precipitated samples, the ease of recovery (defined precipitate vs emulsions) and the ability to fractionally the precipitate material. In our overall work we have selected the IL 1-allyl-3-methylimidazolium chloride ([amim]Cl) for the fractionation experiments, which has been demonstrated to have a good dissolution capability for cellulose and wood materials [7,11-13]. The starting materials were either coarse TMP softwood pulp or sawdust and fine ball-milled powders from soft- or hardwood. In contrast to the usual approach, in component regeneration from IL solutions, we did not use excess of non-solvent caus-

ing rapid precipitation, but gradually increased the amount of a single polar non-solvent to control the amount of precipitated material. By this method, only a fraction of the dissolved material was precipitated, while the rest of the material remained in solution. Careful gel-permeation chromatographic analyses of the fractions offered a visualization of the molecular weight and the distribution of species within the dissolved components. Ball-milled wood dissolved completely in the IL, but the coarse sawdust or TMP pulp preparations were not fully soluble on a microscopic level. It has been noticed earlier that the solubility of softwood in [amim]Cl and subsequent phosphitylation of all hydroxyl functionalities is greatly dependent on the preliminary mechanical treatment[14]. Surprisingly, this partial insolubility of sawdust has enabled a more efficient component separation, by selective extraction of components, compared to the soluble fine powder preparations, which separate according to molecular weight distributions. From the coarse material, a cellulose-rich fraction was extracted and the rest of the lignin-hemicellulose matrix could be isolated as an insoluble material. In the case of increased pulverization, the observed better solubility was rationalized by the fragmentation of the LCC matrix. An increase in the amount of water extractable lignin from crude fractions of milled wood, after the IL treatment, points to the presence of soluble fragments, originating from an LCC matrix.

## **6.2 Advances towards the efficient fractionation of wood with ionic liquids**

Our understanding of the mechanisms operating during fractionation of wood polymers using IL-based solvent systems is continuously improving. An increasing number of publications have appeared in the recent literature utilizing increasingly sophisticated and target-selective treatment systems. The fundamentals of action of the ILs during these treatments have been explored, and the importance of factors such as treatment conditions, solvent system reactivity, and structural features of plant cell walls have all been discussed to varying degrees and will be reviewed in

this chapter. A large number of these citations have focused on the utility of ILs, as pretreatment method prior to biofuel production. Selective separation of the components in these pretreatments may not typically be the ultimate goal of the pretreatment, but frequently delignification takes place. Nevertheless, the publications describing work oriented to increase enzyme activity on wood have offered useful insights into the action of ILs, which can also be utilized for the design of fractionation systems.

#### *New generations of ionic liquids for fractionation applications*

New types of ILs specifically designed to wood fractionation applications have been introduced to the field, and some of the new generations of ILs have also being designed to be more suitable for processing steps, such as recycling of the IL after the treatment. Promising alternatives have been found to the most commonly used dialkylimidazolium acetates and chlorides.

One successful effort that has been made towards more sustainable systems is the development of 'distillable' ILs capable of dissolving cellulose, by King et al.[15] This group of ILs is based on the acid-base conjugates of 1,1,3,3-tetramethylguanidine (TMG) and common carboxylic acids, such as acetic or propionic acid. The distillation ability arises from the fact that the acid-base equilibrium can be shifted to a sufficient extent, at high temperature, to produce volatile neutral species. Yet, the applicability to wood fractionation has not yet been examined. Anugwom et al. have achieved the selective extraction of hemicelluloses using 'switchable' ILs (SIL), that are not capable of dissolving cellulose or lignin from the wood matrix[16,17]. SILs can be formed by reacting CO<sub>2</sub>, and 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) with alcohols. They can be converted back to neutral solvents by removal of CO<sub>2</sub>, under reduced pressure or bubbling with nitrogen. Reversing the IL equilibrium back to volatile components is an interesting potential method for solvent recycling.

In other areas of the IL field, work has been done with the aim of tuning the hydrogen-bonding properties of traditional ILs, to be more selective towards specific wood components. An example of this, by Froschauer at

al.[18] demonstrates modified properties of dialkyl phosphate ILs by using sulfur or selenium to replace one of the oxygen atoms in the anion structure. As a result, the hydrogen-bond accepting ability, as described by the Kamlet-Taft parameter  $\beta$ , was reduced. The new type of anions showed a selective dissolution of hemicelluloses out of hemicellulose-rich pulp. In this regard, Kamlet-Taft parameterization of a range of ILs and co-solvents is starting to allow for a better understanding of solvent and wood biopolymer interactions. In work by Hauru et al., the fundamentals of cellulose dissolution and recovery were presented. They have proposed a different approach to interpret the solvent properties of ILs. Rather than simple evaluation of hydrogen bonding accepting properties ( $\beta$ ), the donor ability ( $\alpha$ ) should be considered as well, resulting in an effective or net basicity value ( $\beta-\alpha$ ) that better describes the cellulose dissolution capability of ILs[19]. Undoubtedly an in-depth understanding of the hydrogen-bonding properties has a key role in sophisticated design of ILs that can selectively extract various main wood components. For lignin isolation, Pinkert et al. have applied dialkylimidazolium sulfamates[20], which do not possess any ability to dissolve cellulose and thus can be used to extract lignin out of biomass. Of note, for this class of IL's, is an earlier application of acesulfamate as a food additive, which makes them promising from an ecotoxicity point-of-view.

#### *Defining the mechanisms of plant cell wall dissolution in first generation ionic liquids*

While the action of new designer ILs on wood is still under investigation, many details about the mechanisms of wood solubility in commonly utilized dialkylimidazolium ILs have been revealed. In the early publications covering the area of wood dissolution, a complete dissolution is mentioned to take place [7,8,12], at least for certain wood species. Since it has been demonstrated that all of the wood components are soluble in ILs in their individual purified forms [5, 21, 22], the naive simplification was made that wood is soluble in such ILs. However, later it was determined

that wood dissolution under mild dissolution conditions was partial, rather than complete. In light of our results and other recent publications, it seems clear that mild dissolutions ( $< 100\text{ }^{\circ}\text{C}$ ) in chloride-based ILs, such as 1-allyl-3-methyl-imidazolium chloride ([amim]Cl), are actually not able to provide the driving force to completely dissolve the wood cell wall, in its native state[14,23-25].

Even if each of the polymeric components, in their purified form, has good solubility in the selected ILs, this does not ensure efficient solubilization of intact fibers. This is because of the complexity of the native wood. The structure of the plant cell wall is complex and highly orientated with many physically and chemically distinct regions, such as primary and secondary (S1, S2, and S3) cell walls and the presence of middle lamella. The variety of polymeric backbone structures, the presence of multiple functionalities and in particular the covalent/physical interactions between the three main wood components make things considerably more complicated. Nature's design of the cell wall is resistant towards physical stress and controls the diffusion of fluids inside the fiber. Inefficient mass transfer of IL or solvated polymers can greatly hinder the dissolution process, even in ILs having high capability for dissolution[26]. This is true especially in the cases where single components are isolated by an extraction type of mechanism. For ILs unable to dissolve cellulose, delignification has been observed to take place mainly on the outer surface of the fibers, due to low accessibility inside the bulky secondary wall[27]. This is analogous to kraft pulping where initial delignification occurs on the fiber surface in the middle lamella and lumen. The different cell types in corn stover show drastic differences in relative lignin solubility, during IL treatment, which further demonstrates the significance of cell wall ultrastructure or composition [26]. Even the early- and late woods in the same sample piece of Sugi wood showed very different response to swelling in IL [28]. They showed that the empty lumen allows for flexibility that protects the fiber from swelling induced physical defects. There also seem to be fundamental differences in the overall solubility, or at least in the kinetics of the dissolution, between soft- and

hardwood species[6,8,24]. Apparently the natural design of tracheids and fibers between soft- and hardwood offers different resistance towards dissolution. All these reports demonstrate that there is a need to focus on revealing the degrees of recalcitrance, related to physical (macroscopic cell wall structure and crosslinking) and chemical factors (polymer interactions and covalent bonding) prior to making generalizations about the efficacy of fractionation of a single IL system on a single species.

Evidently the distribution and structure of lignin in cell walls has a crucial effect on wood solubility. This is not surprising as lignin is considered to be a branched polymer formed by random radical driven crosslinking, thus resembling a complex networked structure[29]. A fundamental property of crosslinked polymer structures is their inability to form true solutions. There are several publications about lignin isolation from ligno-cellulose via extraction-type mechanisms, using various ILs[9,10,20,30,31]. It is also not surprising that depolymerization of the isolated lignins have been observed [9,20,31]. Depolymerization can be a result of several mechanisms, including covalent bond scission via pulverization operations. This can be controlled by the conditions and choice of IL, as discussed in the following section.

The incomplete solubility of cell wall in an unreactive IL does not rule out component fractionation. It seems that dialkylimidazolium chlorides have a much lower reactivity with lignin than other commonly used IL types, such as dialkylimidazolium acetates. A group of publications has shown that this property can be utilized in isolation of cellulose rich fractions[14,23-25]. In particular hemicelluloses and lignin are not separated completely in such systems. Physical or chemical methods to alter cell wall ultrastructure and polymeric networks, prior to or simultaneously with dissolution, may be crucial for complete fractionation. Support of this hypothesis can be found from studies where lignin structure was altered by (1) excessively heating the mixtures until thermal decomposition reactions start to take place[32], or (2) use of an oxidative catalyst to partially degrade the lignin polymer backbone[33]. Enhanced separation of lignin from polysaccharide was achieved, compared to a previous meth-



od[8]. What is not known and not taken into account are the above enumerated considerations and the molecular weight distributions of the resulting pulps.

#### *Role of ionic liquid reactivity in the dissolution of wood*

Alterations to the polymeric structures, arising from reactions where ILs act as catalysts or even a reactive species to form adducts, may have an important role to play for the complete solubility of the composite structures of plant polymers. Recently a number of publications have demonstrated various types of reactivities for ILs, which were at first commonly thought to be relatively inert (with the dialkylimidazolium acetates being a prime example), under the conditions that are typically used in biomass treatments. These results may explain the observed differences between many ILs classes when it comes to mechanisms of cell wall dissolution.

Acid catalyzed reactions seem to be detrimental for both carbohydrates and lignin. Both of these wood components have been shown to partially depolymerize during acidic IL treatments[41]. These include reactions where the presence of the acids in the systems is intentional and where acid has originated from impurities or side reactions, at elevated temperatures. One destructive dissolution IL class are the so-called protic ILs, such as 1-H-3-alkylimidazolium ILs. Cox et al. have found this IL class to readily hydrolyze the  $\beta$ -O-4 ether bonds, in model compounds. Yields were found to be dependent on the anion in the protic IL[34]. The same was also observed with isolated oak wood lignin under conditions above 110 °C[35].

Similar reactions to those observed with protic ILs, degradation has been found to take place even in aprotic ILs, that shouldn't in theory contain significant concentrations of acidic protons. At a temperature of 120 °C, Kubo et al. have found[36] that  $\beta$ -O-4 type model compounds to undergo elimination at the  $\alpha$ - $\beta$ -position[36]. In two chloride based ILs, 1-butyl-3-methylimidazolium chloride ([bmim]Cl) and 1-allyl-3-methylimidazolium chloride ([amim]Cl), enol ether formation, without cleavage of the  $\beta$ -O-4

bond, was the predominant reaction. Part of the original structure was left intact after 72 h incubation. Conversely, in acetate IL 1-ethyl-3-methylimidazolium acetate ([emimAc]) all of the original  $\beta$ -O-4 structures were gone after 72 h, forming only a low amount of enol ethers and the majority of materials other unidentified structures. In agreement with the aforementioned data, George et al.[37] have found that acetate and sulfate ILs dramatically reduce the molecular weight of organosolv lignin, while only mild changes were observed with several chlorides tested under the same conditions. Anyhow, based on the mechanism suggested by Cox et al., even in the chloride ILs the formed enol or vinyl ethers may further hydrolyze in acidic environment in the presence of trace moisture levels and lead to depolymerization[34]. Formation of condensed structures in lignin have also been detected by HSQC-NMR after [emim][OAc] treatment at 155 °C.[38]

There is a notable difference in depolymerization between different lignin preparations, such as alkali and organosolv lignins, where the  $\beta$ -O-4 type of linkages seem to be among the most reactive linkages[37]. Based on structural features it may be reasonable to expect lower reactivity of softwood lignin, compared to hardwood lignin, as softwood lignins have a higher abundance of condensed linkages and hardwoods are rich in the more labile  $\beta$ -O-4 ethers[39].

Impurities remaining from the IL synthesis are likely catalysts for certain reactions. According to Li et al.[32], the delignification of bagasse in [emim][OAc], at temperatures reported to be above the lignin glass-transition temperature, is not happening at even near the same efficiency when recycled IL is used. Loss of the ILs delignification capability, after recycling[13], may allude to the presence of reactive species in the IL as an impurity, which are consumed during the first treatment step.

Cellulose is known to be labile towards acid hydrolysis in the dissolved state. The IL environment has been demonstrated to be effective for such reactions[40,41]. The fact that rapid depolymerisation can also take place in technical ILs, even without added acid catalyst, should be surprising.

Gazit and Katz have demonstrated that cellulose hydrolysis can happen under relatively mild conditions, in commercial-grade dialkylimidazolium chlorides and acetates[42]. The higher purity grades of ILs were also degrading cellulose even faster than lower grades. The catalyst for the reaction was found to be a trace amount acid, formed during the treatment. This could be scavenged by 1-methylimidazole, which is a very typical impurity in low grade commercial ILs. Such observations about acid formation strongly suggest the use of mild temperatures in IL treatments.

Fine control of temperature and dissolution atmosphere may be necessary to reduce the depolymerization of wood during the treatments. According to Miyafuji et al., the depolymerization of carbohydrates in 1-ethyl-3-methylimidazolium chloride ([emim]Cl) can be mostly prevented using temperatures below 90 °C[43]. On the other hand, the use of mild conditions effects negatively to the degree of wood dissolution. Lignin showed much higher resistance towards degradation and any low molecular weight fragments were observed only at temperatures above 110°C. Reactions in chloride-based ILs, induced by atmospheric impurities, have been investigated by Nakamura et al.[44]. Oxygen was found to facilitate the dissolution of lignin, in addition to solubilization of carbohydrates.

Depolymerization is not the only reaction type taking place when lignocellulose is treated with ILs. Addition of dialkylimidazolium cations, at the C2 position, to the reducing ends of polysaccharides has been reported from acetate ILs[45,46-47]. The same effect has been observed with isolated lignin[48]. The reaction follows from self-deprotonation of the imidazolium cation, forming a carbene. The carbene formation and following reactions with electrophiles will lead to conversion of anions to their conjugate acid form[49,50]. Acid species formed in these types of reactions are suggested to be responsible for the depolymerization of cellulose[42], but basic impurities may also capture the released proton. Alternatively, acetylation of cellulose by the IL anion has been observed to happen to low degree in pure [emim][OAc] at high temperature (150 °C)[47]. The mechanism of formation of such structures is still controversial. Çetinkol et al.[51] have reported deacetylation of hemicelluloses and acetylation

of lignin taking place when wood is treated in [emim][OAc]. This suggests a transacetylation mechanism[49,50], but it is still unproven and alternative mechanisms may prevail.

Reactivity of ILs with wood polymers is certainly an important issue related to green processing of wood in such media. Not only for its effects to recycling and atom economy of the processes, but also to the yield, purity, and overall quality of the resulting materials. The use of mild conditions and possible additives, or co-solvents, in IL systems may help to gain control over unwanted side reactions.

#### *Use co-solvents in ionic liquid based dissolution systems*

Research has been performed on the use of organic co-solvents in ILs. This is mainly to alter the properties of the dissolution system, such as viscosity reduction. As protic solvents like water or alcohols tend to prevent cellulose dissolution and are working as efficient non-solvents for dissolved components, the group of polar aprotic organic solvents typically will not decrease the solvation efficiency of ILs towards cellulose, in co-solvent concentrations up to 50 m%[52,53]. The use of co-solvents may enhance the kinetics of the dissolution process, by accelerated diffusion. This allows the use of lower dissolution temperatures that in turn prevent the unwanted depolymerization reactions. Enhancement of wood dissolution kinetics when using co-solvents, compared to pure IL, can be notable, as demonstrated by Qu et al., who aided dissolution of milled Fir with pyridine and DMAc (as co-solvents), at the low temperature of 30 °C[54]. However, much longer dissolution times were needed than for the typical high temperature dissolution. Co-solvents can also enhance wood dissolution at higher temperatures. An article by Xie et al. has demonstrated that complete dissolution of corn stover can be achieved using NMP:[emim][OAc] solutions at the higher temperature of 140 °C, in under 60 min[55]. This of course is not designed for material production, where molecular weights are maintained, but rather biofuel production where maintaining molecular weights is not critical. The majority of the available co-solvents will eventually turn into non-solvent when a limiting concen-

tration is reached[52] and so it may even be possible to maintain binary solvent mixtures with ILs throughout the process. In fractionation processes aiming at the manufacture of derivatized products, certain co-solvents can also act as catalysts for subsequent modification reactions without need for product isolation, in between the unit processes.

Water in the IL systems may also be termed as a limiting solvent, instead of a non- or co-solvent. It has been used as a way of limiting cellulose solubility in certain ILs, while close to complete delignification with removal of hemicelluloses can still take place. The presence of acidic species have been stated to be essential for delignification in these kinds of systems and they can be added as catalysts[56] or originate from the natural acidity of the IL[57]. Depending on the anion of IL, the aqueous solutions can be relatively acidic[56,58]. It remains uncertain whether this is related to impurities specific to pure ILs, technical preparations of ILs, as a natural property of IL-water solutions[58], or from reactions leading to acidic products. Zhang et al. have reported that from a neutral pH of the pre-treatment solvent down to pH 3.4, all of the wood components are regenerating close to their natural compositions from the aqueous IL-system, without resulting in delignified pulp[56]. This is in slight contradiction with the results from Fu et al., who have used neutral aqueous solutions to basic [emim][OAc] efficiently, without any added acid catalysts[59]. The fibrous structure of wood cells still exists in the solid cellulose enriched fractions afforded by treatments in aqueous-IL solutions[56], resembling traditional chemical pulps. This is due to the inability of ILs to dissolve crystalline cellulose, once high enough water contents are added. Thus, only the amorphous parts of the fiber are accessible to the acidic solvent.

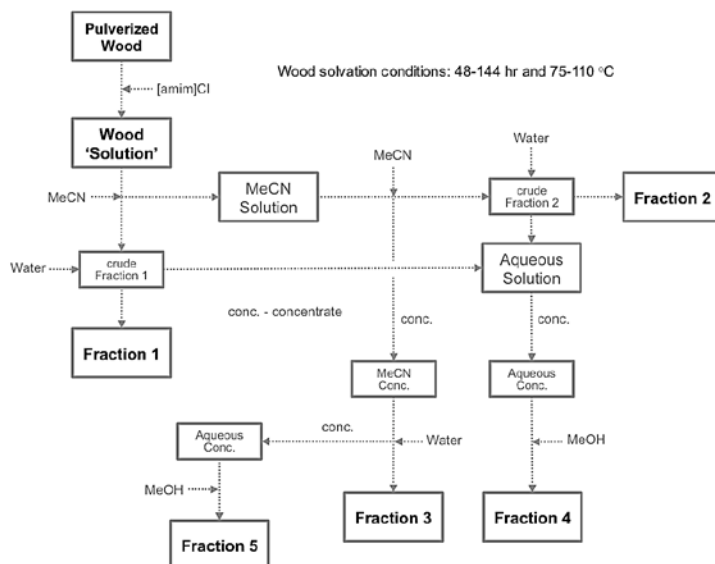
The efficiency of the IL-water solvent system was highly dependent on the type of treated biomass as grass-type feedstocks, such as Miscanthus or Triticale, were found to be highly responsive. Nearly complete delignification and glucose digestibility are observed for grasses, followed by mediocre efficiencies for hardwoods and significantly lower response for softwoods[56,57].

### **6.3 Fractionation of wood by solvation in [amim]Cl and fractional precipitation with a non-solvent**

In this section of the chapter we report data of the fractionation work that was performed in our laboratories. The original focus of our work was to study the mild fractionation and the molecular weight distributions of the resulting precipitated fractions. The reasoning for this was to assess whether it was possible to get technically useful fractions, with suitable molecular weight distributions, by avoiding depolymerisation. Furthermore, the interaction of wood biopolymers and how this affects fractionation, is always a fundamental question that needs answering. [Amim]Cl was used as the solvating IL. It has a low tendency to react with lignin, whilst being an efficient solvent for isolated preparations of all wood components. In agreement with earlier results from our laboratory, King et al.[14], it was found that only heavily pulverized starting materials were completely soluble in the IL of choice. The use of coarse materials, such as sawdust, only offered partial solubility. The reasons behind the solubility differences will be discussed in more detail in this chapter. For the regeneration of dissolved components we have applied an alternative method for the non-solvent addition. For this we have used gradual increases in non-solvent volume instead of rapid excessive addition to the IL-solution. As a result, the regeneration event is well controlled and follows the principles of traditional molecular weight distribution-related polymer fractionation. By applying a derivatization procedure developed in our laboratory, Zoia et al.[60], we have been able to obtain soluble lignocellulose derivatives for size-exclusion chromatography (SEC). This has allowed us to characterize the total molecular weight distributions for majority of the precipitated fractions. Combining the molecular weight information with composition analysis, (acid soluble lignin analysis and IR-spectroscopy), we have been able to observe two fundamentally different mechanisms that apply during component separation, related to the degree of interaction of the wood biopolymers. These are found to be dependent on the degree of pulverization (from extensive milling) and therefore the solubility of the resulting materials. As mentioned previous-

ly solubility of wood is dependent on the degree of pulverization. This therefore influences whether the fractionation is extraction based or solvation (and subsequent selective precipitation) based. An acetonitrile non-solvent was able to regenerate majority of the dissolved materials but additional non-solvents, such as water and methanol, allowed for further component separation, based on further chemical composition. The motives for selecting this non-solvent, and more comprehensive discussion about our data can be found from our earlier publications[23,61]. It was also found that further purification of isolated crude fractions with water resulted in secondary separations and it was possible to recover more water-soluble materials in their own fraction. A complete flow diagram of the fractionation scheme is presented in figure 1. Selected starting materials (see table 6.1) have undergone different mechanical pretreatment processes. Particle sizes and properties changed accordingly with the preparation method. Nearly intact fibrous structures have remained after TMP pulping, but were notably fragmented during sawdust preparation. Ball-milled materials represent highly pulverized wood that has lost all fibrous characteristics.

As mentioned previously, fractions were analyzed by Klason lignin analysis, ATR-IR and the molecular weight distributions of some fractions were determined by re-dissolution into [amim]Cl, benzylation and SEC analysis (Zoja et al.[60]). The analysis results are presented in Table 6.2.



**Figure 6.1** Total fractionation procedure. Fraction 5 was not isolated in every experiment. Reprinted with permission from [23b]. Copyright © 2013 American Chemical Society.

The fractionation procedure was performed roughly as follows: Wood samples were heated with [amim]Cl for the specified period and temperature. Crude fraction 1 was precipitated from IL using acetonitrile as non-solvent and was washed with water and dried. Fraction 2 was precipitated from the residual IL solution by addition of further acetonitrile and further washed, using the same procedure as for fraction 1. Fraction 3 was precipitated from IL-solution by water addition, after the acetonitrile had been removed by evaporation. Fraction 4 was prepared from the combined aqueous extracts from fractions 1 and 2. The aqueous extracts were combined, concentrated, precipitated with methanol and dried. Fraction 5 was prepared by concentration of the remaining water solution, from fraction 3, precipitation with methanol and drying.



**Table 6.1** Starting materials, their upper particle diameter limits, and lignin contents. \* = Value from literature[62]

<b>Material</b>	<b>Particle diam.</b>	<b>Lignin content</b>
Wiley milled Norway spruce TMP pulp	< 400 $\mu\text{m}$	28.7 %
Norway spruce sawdust	< 200 $\mu\text{m}$	26.6 %
Ball milled Norway spruce TMP pulp	< 75 $\mu\text{m}$	28.7 %
Ball milled <i>Eucalyptus grandis</i>	< 75 $\mu\text{m}$	25.8 %*

**Table 6.2** All fractions collected in this study including yields, lignin contents and analyses performed. Fraction 5 is not included in the table or in yield calculations.

<b>Starting materials and conditions</b>	<b><math>Y_{total}</math> [%]</b>	<b>Fraction number</b>	<b><math>Y_{Fraction}</math> [%]</b>	<b>Lignin cont. [%]</b>	<b><math>Y_{Lignin}</math> [%]</b>	<b><math>Y_{Carb.}</math> [%]</b>
[amim]Cl 6 % 40 mesh TMP spruce 144 h 100 °C	86	1	57.4	37.7	75	50
		2	25.9	12.1	11	32
		3	0.5	-	-	-
		4	1.8	-	-	-
[amim]Cl 4 % spruce sawdust 120 h 110 °C	93	1	53.2	45.0	90	40
		2	33.2	6.2	8	42
		3	1.3	-	-	-
		4	4.8	-	-	-
[amim]Cl: 10 % 28 days rotary-milled TMP spruce 48 h 75 °C	86	1	45.7	28.4	45	46
		2	21.6	36.5	27	19
		3	2.4	-	-	-
		4	16.3	20.9	12	18
[amim]Cl: 10 % 48h ball-milled eucalyptus	59 <sup>#</sup>	1	42.1	9.0	15	52
		2	12.7	-	-	-

48 h 75 °C

3

4.2

-

-

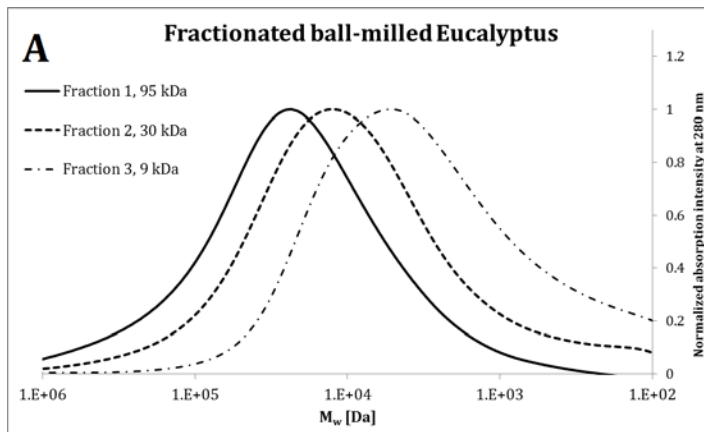
-

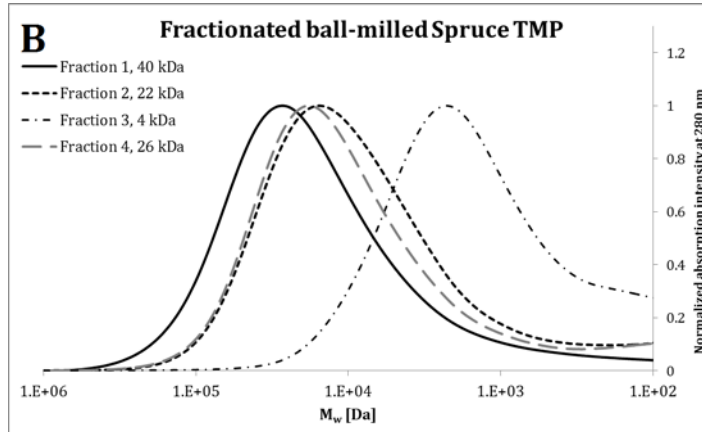
# = Fraction 4 not included.  $Y_{total}$  = Total yields of precipitated material.  $Y_{Fraction}$  = Yield of precipitated fraction from starting material, **Lignin cont.** = Lignin content of fraction that includes Klason lignin + acid soluble lignin,  $Y_{Lignin}$  = Yield of lignin in fraction from total lignin content in starting material,  $Y_{Carb.}$  = Yield of carbohydrates in fraction including cellulose and hemicelluloses, - = Value not determined. Reprinted with permission from [23b]. Copyright © 2013 American Chemical Society.

### 6.3.1 Fractionation based on molecular weight

It is well known that polymers of different molecular weight have different solubility in solvents. This means that controlling the precipitation of polymers, of high polydispersity, from any solution can be used to separate them into fractions of decreasing molecular weight[63]. The main components in wood have distinctively different average degrees of polymerization (DP). Isolated softwood celluloses have been measured to have average molecular weights from 730 kDa[64] up to 1550 kDa[65]. Hemicelluloses are of typically lower DP than cellulose and isolated hemicelluloses consist of polymers on average from 18 to 80 kDa[66-67], depending on the isolation method. Lignin preparations that represent as close to native lignin as we can isolate with current methods, have molecular weights between 52 to 98 kDa[68]. Differences of such magnitude, including differences in chemical composition of the polymers, should offer plenty of opportunity for separation of lignin from hemicellulose from cellulose by controlled addition of a nonsolvent into ionic liquid. Articles by Lee et al. and Lateef et al.[10,69], have shown that mixtures of the purified polymers can be highly selectively precipitated from IL solutions. In actual fractionation processes, using minimally treated wood, this efficiency is never observed.

If we examine at the molecular weight distributions of the fractions from the highly pulverized, 28 days rotary milled and 48 h ball-milled, samples from spruce and Eucalyptus respectively (Figure 6.2), we can see that there is a distinct precipitation based on molecular weight. In both cases, the molecular weights of the fractions decrease from fractions 1 to 3. Fraction 4 overlaps with fraction 2 for spruce, due to the fact that the majority of the material in the water-soluble fraction was originally dissolved from crude fraction 2 (see figure 6.1). If we look at the lignin contents for the main fractions 1 and 2 for spruce (see Table 6.2 entry for milled TMP), there is very little change in the lignin contents from the native wood. Thus there is clear evidence for precipitation based on molecular weight and very poor separation of lignin from polysaccharide, contrary to previous reports on the separation of mixtures of the purified polymers[10,69]. It seems evident that lignin-carbohydrate complexes (LCCs) are preventing the separation of the lignin and polysaccharide portion of this fully soluble pulverized wood. Seemingly, disintegration of the LCC matrix during pulverization creates fragments that have similar molecular weights to cellulose, and that have been extensively depolymerized during milling. As a result, a mixture of similar sized LCC polymers precipitate in order of molecular weight.





**Figure 6.2** The molecular weight distributions of the isolated fractions, from fully soluble ball-milled wood. The regeneration order was largely controlled by the molecular weight of the components. Fractions from A) milled Eucalyptus B) milled Spruce TMP

### 6.3.2 Effect of particle size on fractionation mechanisms

For fully soluble finely pulverized materials, the dissolution is relatively rapid for all components. Dissolution is much faster compared to coarse materials and wood converts to a completely solvated state. Solvation was not complete with the coarse materials. The following dissolution and fractionation mechanism seemed to differ greatly from the pulverized materials, based on compositional analyses of isolated fractions (table 2) and XRD-analysis of the sawdust fraction 1 and the original sawdust (figure 6.3).

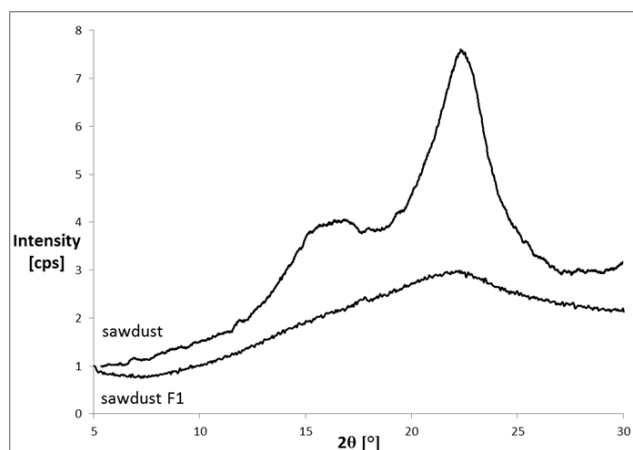
For the incomplete dissolution of the coarser materials (sawdust and TMP), fraction 1 was mostly composed of the materials that remained solid (but seemingly swollen) during the whole dissolution/extraction period. This was determined to be mainly lignin and polysaccharide. Fraction 2 was determined to be mainly cellulose, based on Klason lignin and ATR-IR analyses (Figure 6.4). As fraction 2 was ~33 % of the original sawdust frac-

tion, meaning that most of the cellulose is extracted from the insoluble wood sample leaving an insoluble matrix of lignin and hemicellulose. Further evidence of this is found after XRD analysis of the regenerated fraction 2, in comparison to the starting sawdust. After extraction of cellulose, the amorphous LCC network was remaining. One should ask the questions, 'Why is there an insoluble fraction when the purified polymers are all soluble in the IL?' and 'Why can lignin not be separated efficiently from the polysaccharide, even when the finely pulverized samples are completely soluble?'.

Both questions can be addressed by the explanation of precipitation based on molecular weight. However, most solvents will preferentially precipitate one component over another and this is simply not the complete picture. Both questions can be answered if you take in to consideration that wood is suggested to contain LCCs and it is actually the LCC network that is preventing dissolution. Only material that is not bound into the LCC network is extractable, under mild dissolution conditions. This is entirely consistent with a report by Lawoko et al.[70] showing that almost all isolatable LCCs from Norway spruce consist of lignin, which is chemically bonded with hemicelluloses. Whereas, only a minor portion of spruce LCCs have been found to contain lignin-cellulose type LCCs. With this literature confirmation it is no wonder that we can extract cellulose from an insoluble LCC matrix.

As anticipated, it was nearly impossible to derivatize and dissolve any further material from fraction 1 for SEC, due to its insolubility. In case of highly pulverized wood, physical degradation of all polymeric components seem to take place and overall polydispersity decreases. Fractions from ball milled TMP pulp further gave more evidence about the close association with lignin and carbohydrates and how these structures could control the total dissolution of wood. The FT-IR analysis offered some details about the carbohydrate compositions in isolated fractions. Neutral sugar analysis is traditionally used to characterize the carbohydrate moieties in lignocellulose, but in this work FT-IR was used instead as a fast, non-destructive, and semi-quantitative analytical method. IR spectra of the

isolated fractions from two spruce materials, ball-milled TMP and sawdust, are presented in figure 4. When spectra of fraction 1 from the two materials are compared, the significant presence of hemicelluloses can be seen for sawdust, whereas in milled TMP the carbohydrates seem to be mostly cellulose. The hemicelluloses are more present in the lower molecular weight fraction 2 for the case of milled TMP. Other significant differences can be found in composition and yield of the water-soluble fraction 4. For sawdust it seems that the majority of the hemicelluloses have remained totally water insoluble in fraction 1, for some yet unknown reason. For milled wood around half of the original hemicelluloses were converted to be water soluble and dissolved together with relatively large portions of lignin. No lignin was observed to be water soluble from sawdust crude fractions.

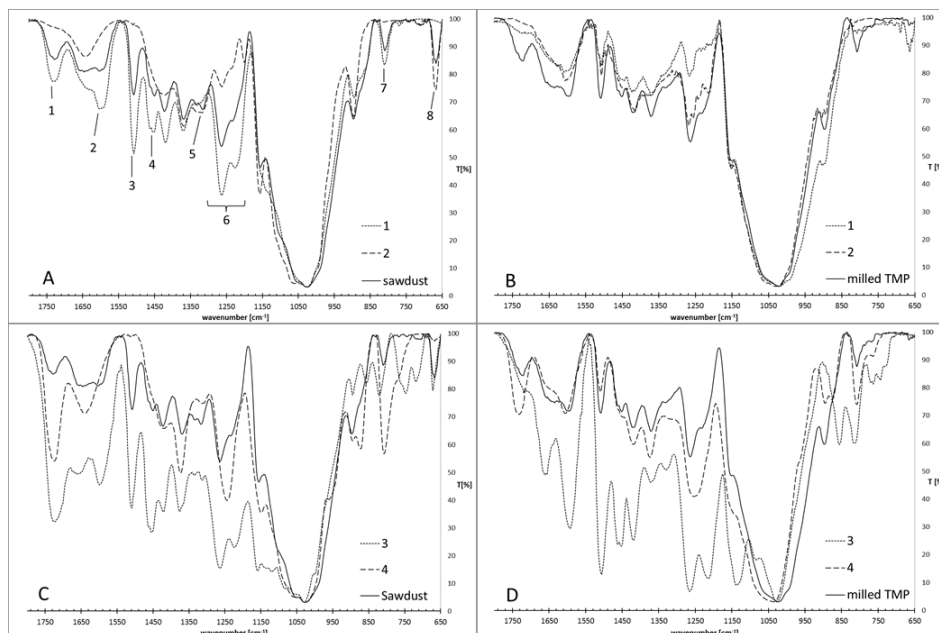


**Figure 6.3** X-ray powder diffractograms of spruce sawdust, starting material, (above) and fraction 1, recovered from [amim]Cl. Reprinted with permission from [23b]. Copyright © 2013 American Chemical Society.

Once again, this observation could be explained by the covalent attachment of hemicelluloses that are released during the milling via the fragmentation of the supporting lignin polymers, that otherwise would prevent them from being extracted during the water washing.

Sawdust and TMP pulp preparations represent structurally quite unaltered wood. Our results suggest that swelling and dissolution of native or relatively intact fibers start from the amorphous and crystalline domains of cellulose. The solvated cellulose polymers then diffuse to the bulk solution (*fraction 2*) leaving behind the lignin-hemicellulose matrix that remains in a rather swollen form and is restricted from complete dissolution (*fraction 1*). Only minor fractions of lignin (*fraction 3*) or hemicelluloses (*fraction 4*) seem to be unbound and transfer to bulk solution. Molecular weight analysis showed that the isolated cellulose-rich fraction 2 had a significantly lower molecular weight than reported for e.g. softwood pulps.

From the dissolution treatment, it is hard to estimate if there is significant depolymerization during the 48 h dissolution period. For the sawdust fractionation, some depolymerization of the carbohydrate components during the dissolution seems evident. Molecular weight of the isolated fraction 2 is low, considering the fact that this fraction was composed mostly of cellulose, as our analysis revealed (see Table 6.2 and figure 6.4). In light of a recent study by Gazit and Katz[42], the depolymerization of cellulose during long dissolution periods, even in purified IL, is not surprising. Their results indicate that trace level formation of acidic by-products can take place during dissolution, even below the temperatures that were used in our work. The unfortunate fact is that technical pulps have very defined specifications, in terms of molecular weight distributions, and not only their lignin and hemicellulose contents. This means that controlling acidic and oxidative impurities during an IL-mediated fractionation will be critical in the future to obtain technically useful pulps that fit existing value-chains. In many cases even the present commercial ILs contain these impurities. More must be done to quantify and understand the effects of these impurities.



**Figure 6.4** FT-IR spectra of fractions 1-4 precipitated 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, D) Milled TMP fractions 3 and 4. **Band assignments:** 1) Carbonyl groups from hemicelluloses and lignin[71,72] 2) Carboxylic acids from xylan and lignin[71,72] 3) Lignin[73] 4) Xylan[72] 5) Cellulose[73] 6) Carbonyl groups from hemicelluloses and lignin[72] 7) Glucomannan[74] 8) Cellulose[73]. Reprinted with permission from [23b]. Copyright © 2013 American Chemical Society.

The single experiment that was performed with Eucalyptus resulted in a lignin poor fraction that was the first to precipitate from the IL-solution (*fraction 1*). As mentioned previously, precipitation was dependent on molecular weight for Eucalyptus, as well as milled spruce. It remains as a topic for further studies how differences in covalent structures between lignin and polysaccharide will affect the selectivity of separation. Other ILs like [emim][OAc] have been reported to fully dissolve hardwood[8]. This may be due to chemical degradation of LCC-matrix during the treatment,



as discussed earlier. It remains possible that other ILs could facilitate higher yields of cellulose-enriched materials by gradual precipitation, only if the LCC cleavage and carbohydrate depolymerization remains at a low level during the dissolution.

## 6.4 Conclusions

Due to the rapidly expanding field of IL mediated wood processing, our knowledge in this area has increased to a new level. Many new technical advances are apparent, including more refined ILs, electrolytes, pre-treatments and processing techniques. However, the application of fundamental knowledge related to the connectivity of wood biopolymers, wood morphology, wood ultrastructure and even the solubility of wood in ILs seems to have been largely neglected. Increasing awareness related to IL reactivity has brought both challenges and possibilities to wood fractionation. Depolymerization during fractionation can result in undesired products. This is most relevant when molecular weight distributions should be maintained, e.g. for the production of cellulosic pulps. However, in some cases degradation may be beneficial, e.g for dissolving the LCC network or reducing the recalcitrance of wood for biofuel production.

Based on our work, with sawdust and highly pulverized spruce wood, we have demonstrated that wood is not completely soluble in [amim]Cl in its native state. This is confusing as isolated lignin, cellulose and hemicellulose preparations have been dissolved efficiently in several publications. One possible reason for this is the presence of an extended LCC matrix in wood that is simply of too high molecular weight and interconnected to dissolve. This property can be utilized to extract cellulose, as it is not covalently bound to the insoluble LCC matrix. Cellulose is extracted and by careful control of non-solvent addition, the insoluble lignin-hemicellulose rich fraction can be first isolated, followed by regeneration of relatively pure cellulose. This cellulose extraction procedure is not yet at a stage that would yield a technically useful pulp, due to apparent depolymerization, in comparison to technical pulps and holo-cellulose. However, the more we learn about the stability of wood and lignocellulose, in technical

and pure ILs, the better are our chances of yielding close-to native polymers.

The implications for biofuels production are more straight-forward, in regard to pre-treatment mechanisms. Certain ionic liquids are excellent media for cellulose dissolution and regeneration to a state, which is easier to process. Presence of impurities or intentionally added catalysts, that may depolymerize the biopolymers during this process, are beneficial, provided the IL is somewhere between 99-100 % recoverable. This is a function of the high cost of ILs, at present. The method of biopolymer regeneration, to enhance separate lignin from polysaccharide, is therefore quite important. If degradation is significant enough breakage of covalent linkages between lignin and polysaccharide should facilitate this. IL recyclability is a major challenge here due to the buildup of monomers, dimers, oligomers, silicates and other inorganics. Therefore improving IL recyclability will greatly enhance the chances of success.

## 6.5 Experimental

### *Materials*

Synthesis of the [amim]Cl was performed according to a method adapted from Wu et al[75]. 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 <sup>1</sup>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 [amim]Cl as a pale yellow viscous oil. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 3.97 (3H, s, NCH<sub>3</sub>), 4.86 (2H, d, J = 6.4 Hz, NCH<sub>2</sub>),

5.33-5.26 (2H, m, C=CH—C), 5.86 (1H, ddt,  $J = 16.9$  Hz,  $10.3$  Hz,  $6.5$  Hz, C=CH<sub>2</sub>), 7.40 (1H, s, C=CH), 7.65 (1H, s, C=CH), 10.39 (1H, s, NCHN).

Unbleached Norway spruce (*Picea abies*) thermomechanical pulp (TMP) was donated from a Swedish mill. Norway spruce sawdust (particle size < 0.2 mm by sieving) was prepared with a belt grinder (grade 60), in-house. *Eucalyptus grandis* was supplied by Novozymes, NC, USA. 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 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  $\mu$ m).

Eucalyptus chips were Soxhlet extracted with acetone for 48 h. Remaining tannins were removed by refluxing in 0.075M NaOH solution (1:50 w/v ratio) for 1 h prior to milling of the dried sample. Milling was performed in a Fritsch Pulverette planetary ball-mill, 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 of the sample. All the wood materials were dried in vacuum oven over night at 40 °C prior to their use.

***Solvation of wood with [amim]Cl***

Lignocellulosic material (typically *ca.* 1 g) was quickly added to a flask containing dry [amim]Cl (typically *ca.* 20g) 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, ranging from 48 h at 80 °C to 122h at 110 °C. Rotary milled Norway spruce powder generally dispersed and gave 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 non-solvent addition***

Preparation of fractions 1 to 4 was carried out as follows; Crude fraction 1 was precipitated from IL using acetonitrile as non-solvent. The crude fraction was separated using centrifuge and washed with water, so that filtrate was retained. The solid residue was dried to give fraction 1. For preparation of fraction 2 additional acetonitrile was added into IL-solution followed by similar separation and washing procedure. Fraction 3 was precipitated from IL-solution by water addition, after the acetonitrile had been concentrated by evaporation. Fraction 4 was prepared from the aqueous filtrates retained from the purification of fractions 1 and 2. Filtrates were concentrated down to a volume *ca.* 2 ml, which was followed by addition of MeOH. Formed fluffy precipitate was filtered and purified with MeOH. Fraction 5 was prepared as follows. The aqueous IL-solution left from the precipitation of fraction 3 was concentrated so that nearly all water had removed. The remaining IL-solution was precipitated with MeOH (1:10 v/v). Formed precipitation was filtered in grade-3 sinter, washed with MeOH and the filtrand was dried in a vacuum oven for 18 h at 40 °C to give fraction 5.

### ***Analysis of precipitated fractions***

For molecular weight determination the fractionated samples were derivatized with benzoyl chloride in [amim]Cl solution following the procedure introduced by Zoia et al.[60] and analyzed using HP G1312A pump connected to Waters HR5E and HR1 columns with a Waters 484 UV-absorbance detector calibrated using polystyrene standards. Acid insoluble (Klason) lignin and acid soluble lignin were determined by method modeled from one published by Dence[76]. The acid that was used to hydrolyze the samples was diluted from conc. sulfuric acid corresponding to  $72 \pm 0.1$  %. The lignocellulose samples were dried in a vacuum oven at 40 °C overnight. *ca.* 100 mg of the samples were measured accurately and mixed with sulfuric acid solution (100 mg per 2 ml) using magnetic stirring and vortex mixer. After 2 hour 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 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 pathlength cuvette. The concentrations were calculated using extinction co-efficient of 110 l/g\*cm.

FT-IR spectra were recorded from finely powdered samples that were dried for 20h at 50°C in a vacuum oven, using 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.

## References

1. Sun N, Rodriguez H, Rahman M, Rogers R D (2011) Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? *Chem. Commun* 47:1405-1421.
2. Mäki-Arvela P, Anugwom I, Virtanen P, Sjöholm R, Mikkola J P (2010) Dissolution of lignocellulosic materials and its constituents using ionic liquids—A review. *Industrial Crops and Products* 32:175-201.
3. Mora-Pale M, Meli L, Doherty T V, Linhardt R J, Dordick J S (2011) Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnol. Bioeng.* 108:1229-1245.
4. Tadesse H, Luque R (2011) Advances on biomass pretreatment using ionic liquids: An overview. *Energy Environ. Sci.* 4:3913-3929.
5. Swatloski R P, Spear S K, Holbrey J D, Rogers R D (2012) Dissolution of Cellulose with Ionic Liquids. *J. Am. Chem. Soc.* 124:4974-4975.
6. Fort D A, Remsing R C, Swatloski R P, Moyna P, Moyna G, Rogers R D (2007) Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. *Green Chem.* 9:63-69.
7. Kilpelainen I, Xie H, King A, Granstrom M, Heikkinen S, Argyropoulos D S (2007) Dissolution of Wood in Ionic Liquids. *J. Agric. Food Chem.* 55:9142-9148.
8. Sun N, Rahman M, Qin Y, Maxim M L, Rodríguez H, Rogers R D (2009) Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. *Green Chem.* 11:646-655.
9. Tan S S Y, MacFarlane D R, Upfal J, Edey L A, Doherty W O S, Patti A F, Pringle J M, Scott J L (2009) Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chem.* 11:339-345.

10. Lee S H, Doherty T V, Linhardt R J, Dordick J S (2009) Ionic Liquid-Mediated Selective Extraction of Lignin from Wood Leading to Enhanced Enzymatic Cellulose Hydrolysis. *Biotechnology & bioengineering* 102:1368-1376.
11. Zhang H, Wu J, Zhang J, He J (2005) 1-Allyl-3-methylimidazolium Chloride Room Temperature Ionic Liquid: A New and Powerful Nonderivatizing Solvent for Cellulose. *Macromolecules* 38:8272-8277.
12. Zavrel M, Bross D, Funke M, Buchs J, Spiess A C, (2009) High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresour. Technol.* 100:2580-2587.
13. Li B, Asikkala J, Filpponen I, Argyropoulos D S (2010) Factors Affecting Wood Dissolution and Regeneration of Ionic Liquids. *Ind Eng Chem Res* 49:2477-2484.
14. King A, Zoia L, Filpponen I, Olszewska A, Xie H, Kilpeläinen I, Argyropoulos D S (2009) In Situ Determination of Lignin Phenolics and Wood Solubility in Imidazolium Chlorides Using <sup>31</sup>P NMR. *J. Agric. Food Chem.* 57:8236-8243.
15. King A W T, Asikkala J, Mutikainen I, Järvi P, Kilpeläinen I (2011) Distillable Acid-Base Conjugate Ionic Liquids for Cellulose Dissolution and Processing. *Angewandte Chemie International Edition* 50:6301-6305.
16. Anugwom I, Mäki-Arvela P, Virtanen P, Damlin P, Sjöholm R, Mikkola J (2011) Switchable ionic liquids (SILs) based on glycerol and acid gases. *RSC Adv.* 452
17. Anugwom I, Mäki-Arvela P, Virtanen P, Willför S, Sjöholm R, Mikkola J (2012) Selective extraction of hemicelluloses from spruce using switchable ionic liquids. *Carbohydr. Polym.* 87:2005-2011.
18. Froschauer C, Hummel M, Laus G, Schottenberger H, Sixta H, Weber H K, Zuckerstätter G (2012) Dialkyl Phosphate-Related Ionic Liquids as Selective Solvents for Xylan. *Biomacromolecules* 13:1973.

19. Hauru L K J, Hummel M, King A W T, Kilpeläinen I, Sixta H (2012) Role of Solvent Parameters in the Regeneration of Cellulose from Ionic Liquid Solutions. *Biomacromolecules* 13:2896.
20. Pinkert A, Goeke D F, Marsh K N, Pang S (2011) Extracting wood lignin without dissolving or degrading cellulose: investigations on the use of food additive-derived ionic liquids. *Green Chem.* 13:3124.
21. Pu Y, Jiang N, Ragauskas A J (2007) Ionic Liquid as a Green Solvent for Lignin. *J. Wood Chem. Technol.* 27:23-33.
22. Peng X, Ren J L, Sun R C (2010) Homogeneous Esterification of Xylan-Rich Hemicelluloses with Maleic Anhydride in Ionic Liquid. *Biomacromolecules* 11:3519-3524.
23. a) Leskinen T, King A W T, Kilpeläinen I, Argyropoulos D S (2011) Fractionation of Lignocellulosic Materials with Ionic Liquids. 1. Effect of Mechanical Treatment. *Ind. Eng. Chem. Res.* 50:12349-12357. b) Leskinen T, King A W T, Kilpeläinen I, Argyropoulos D S, (2013) Fractionation of Lignocellulosic Materials Using Ionic Liquids: Part 2. Effect of Particle Size on the Mechanisms of Fractionation, *Ind. Eng. Chem. Res.* 52:3958-3966.
24. Wang X, Li H, Cao Y, Tang Q (2011) Cellulose extraction from wood chip in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl). *Bioresour. Technol.* 102:7959-7965.
25. Casas A, Alonso M V, Oliet M, Santos T M, Rodriguez F (2013) Characterization of cellulose regenerated from solutions of pine and eucalyptus woods in 1-allyl-3-methylimidazolium chloride. *Carbohydr. Polym.* 92:1946-1952.
26. Sun L, Li C, Xue Z, Simmons B A, Singh S (2013) Unveiling high-resolution, tissue specific dynamic changes in corn stover during ionic liquid pretreatment. *RSC Adv.* 3:2017-2027.
27. Doherty T V, Mora-Pale M, Foley S E, Linhardt R J, Dordick J S. (2010) Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chem.* 12:1967-1975.



28. Miyafuji H, Suzuki N (2012) Morphological changes in sugi (*Cryptomeria japonica*) wood after treatment with the ionic liquid, 1-ethyl-3-methylimidazolium chloride. *J Wood Sci* 58:222-230.
29. Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. *Annu. Rev. Plant Biol.* 54:519-546.
30. Fu D, Mazza G, Tamaki Y (2010) Lignin Extraction from Straw by Ionic Liquids and Enzymatic Hydrolysis of the Cellulosic Residues. *J. Agric. Food Chem.* 58:2915-2922.
31. Kim J, Shin E, Eom I, Won K, Kim Y H, Choi D, Choi I, Choi J W (2011) Structural features of lignin macromolecules extracted with ionic liquid from poplar wood. *Bioresour. Technol.* 102:9020-9025.
32. Li W, Sun N, Stoner B, Jiang X, Lu X, Rogers R D (2011) Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chem.* 13:2038-2047.
33. Sun N, Jiang X, Maxim M L, Metlen A, Rogers R D (2011) Use of Polyoxometalate Catalysts in Ionic Liquids to Enhance the Dissolution and Delignification of Woody Biomass. *ChemSusChem* 4:65-73.
34. Cox B J, Jia S, Zhang Z C, Ekerdt J G (2011) Catalytic degradation of lignin model compounds in acidic imidazolium based ionic liquids: Hammett acidity and anion effects. *Polym. Degrad. Stab.* 96:426-431.
35. Cox B J, Ekerdt J G (2012) Depolymerization of oak wood lignin under mild conditions using the acidic ionic liquid 1-H-3-methylimidazolium chloride as both solvent and catalyst. *Bioresour. Technol.* 118:584-588.
36. Kubo S, Hashida K, Yamada T, Hishiyama S, Magara K, Kishino M, Ohno H, Hosoya S (2008) A Characteristic Reaction of Lignin in Ionic Liquids; Glycelol Type Enol-Ether as the Primary Decomposition Product of  $\beta$ -O-4 Model Compound. *J. Wood Chem. Technol.* 28:84-96.
37. George A, Tran K, Morgan T J, Benke P I, Berruenco C, Lorente E, Wu B C, Keasling J D, Simmons B A, Holmes B M (201) The effect of ionic

liquid cation and anion combinations on the macromolecular structure of lignins. *Green Chem.* 13:3375-3385.

38. Torr K M, Love K T, Cetinkol O P, Donaldson L A, George A, Holmes B M, Simmons B A (2012) The impact of ionic liquid pretreatment on the chemistry and enzymatic digestibility of *Pinus radiata* compression wood. *Green Chem.* 14:778-787.
39. Argyropoulos D, Menachem S (1997) Lignin. Eds.; *Biotechnology in the Pulp and Paper Industry*; Springer Berlin Heidelberg 57:127-158.
40. Rinaldi R, Meine N, Vomstein J, Palkovits R, Schüth F (2010) Which Controls the Depolymerization of Cellulose in Ionic Liquids: The Solid Acid Catalyst or Cellulose? *ChemSusChem* 3:266-276.
41. Li B, Filpponen I, Argyropoulos D S (2010) Acidolysis of Wood in Ionic Liquids. *Ind Eng Chem Res* 49:3126-3136.
42. Gazit O M, Katz A (2012) Dialkylimidazolium Ionic Liquids Hydrolyze Cellulose Under Mild Conditions. *ChemSusChem* 5:1542-1548.
43. Miyafuji H, Miyata K, Saka S, Ueda F, Mori M (2009) Reaction behavior of wood in an ionic liquid, 1-ethyl-3-methylimidazolium chloride. *J Wood Sci* 55:215-219.
44. Nakamura A, Miyafuji H, Saka S (2010) Influence of reaction atmosphere on the liquefaction and depolymerization of wood in an ionic liquid, 1-ethyl-3-methylimidazolium chloride. *J Wood Sci* 56:256-261
45. Liebert T, Heinze T (2008) Interaction of ionic liquids with polysaccharides 5. Solvents and reaction media for the modification of cellulose. *Bioresources* 3:576-601.
46. Ebner G, Schiehser S, Potthast A, Rosenau T (2008) Side reaction of cellulose with common 1-alkyl-3-methylimidazolium-based ionic liquids. *Tetrahedron Lett.* 49:7322-7324.

47. Karatzos S, Edey L, Wellard R (2012) The undesirable acetylation of cellulose by the acetate ion of 1-ethyl-3-methylimidazolium acetate. *Cellulose* 19:307-312.
48. Kim J, Shin E, Eom I, Won K, Kim Y H, Choi D, Choi I, Choi J W (2011) Structural features of lignin macromolecules extracted with ionic liquid from poplar wood. *Bioresour. Technol.* 102:9020-9025.
49. Rodriguez H, Gurau G, Holbrey J D, Rogers R D, Reaction of elemental chalcogens with imidazolium acetates to yield imidazole-2-chalcogenones: direct evidence for ionic liquids as proto-carbenes. *Chem. Commun.* 47:3222-3224.
50. King A W T, Parviainen A, Karhunen P, Matikainen J, Hauru L K J, Sixta H, Kilpelainen I (2012) Relative and inherent reactivities of imidazolium-based ionic liquids: the implications for lignocellulose processing applications. *RSC Adv.* 2:8020-8026.
51. 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 (2010) Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* 1:33-46.
52. Gericke M, Liebert T, Seoud O A E, Heinze T (2011) Tailored Media for Homogeneous Cellulose Chemistry: Ionic Liquid/Co-Solvent Mixtures. *Macromolecular Materials and Engineering* 296:483-493.
53. Rinaldi R (2011) Instantaneous dissolution of cellulose in organic electrolyte solutions. *Chem. Commun.* 47:511-513.
54. Qu C, Kishimoto T, Hamada T, Nakajima N (2012) Dissolution and acetylation of ball-milled lignocellulosic biomass in ionic liquids at room temperature: application to nuclear magnetic resonance analysis of cell-wall components. *Holzforschung* 67:25-32.
55. Xie H, Shen H, Gong Z, Wang Q, Zhao Z K, Bai F (2012) Enzymatic hydrolysates of corn stover pretreated by a N-methylpyrrolidone-ionic liquid solution for microbial lipid production. *Green Chem.* 14:1202-1210.

56. Zhang Z, O'Hara I M, Doherty W O S (2013) Effects of pH on pretreatment of sugarcane bagasse using aqueous imidazolium ionic liquids. *Green Chem.* 15:431-438.
57. Brandt A, Ray M J, To T Q, Leak D J, Murphy R J, Welton T (2011) Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid-water mixtures. *Green Chem.* 13:2489-2499.
58. Zhang Y, Du H, Qian X, Chen E Y (2010) Ionic Liquid-Water Mixtures: Enhanced Kw for Efficient Cellulosic Biomass Conversion. *Energy Fuels* 24:2410-2417.
59. Fu D, Mazza G, Aqueous ionic liquid pretreatment of straw. *Bioresour. Technol.* 102:7008-7011.
60. Zoia L, King A W T, Argyropoulos D S (2011) Molecular Weight Distributions and Linkages in Lignocellulosic Materials Derivatized from Ionic Liquid Media. *J. Agric. Food Chem.* 59:829-838.
61. Casas A, Alonso M V, Oliet M, Rojo E, Rodríguez F (2012) FTIR analysis of lignin regenerated from *Pinus radiata* and *Eucalyptus globulus* woods dissolved in imidazolium-based ionic liquids. *Journal of Chemical Technology & Biotechnology* 87:472-480.
62. Emmel A, Mathias A L, Wypych F, Ramos L P (2003) Fractionation of *Eucalyptus grandis* chips by dilute acid-catalysed steam explosion. *Bioresour. Technol.* 86:105-115.
63. Cantow M J R (1967) Ed.; In *Polymer fractionation*; Academic Press: New York. 527.
64. Yanagisawa M, Shibata I, Isogai A (2005) SEC-MALLS analysis of softwood kraft pulp using LiCl/1,3-dimethyl-2-imidazolidinone as an eluent. *Cellulose* 12:151-158.
65. Schult T, Hjerde T, Optun O I, Kleppe P J, Moe S (2002) Characterization of cellulose by SEC-MALLS. *Cellulose* 9:149-158.

66. Jacobs A, Dahlman O (2001) 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* 2:894-905.
67. Lundqvist J, Teleman A, Junel L, Zacchi G, Dahlman O, Tjerneld F, Stålbrand H (2002) Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydr. Polym.* 48:29-39.
68. Guerra A, Filpponen I, Lucia L A, Saquing C, Baumberger S, Argyropoulos D S (2006) Toward a Better Understanding of the Lignin Isolation Process from Wood. *J. Agric. Food Chem.* 54:5939-5947.
69. Lateef H, Grimes S, Kewcharoenwong P, Feinberg B (2009) Separation and recovery of cellulose and lignin using ionic liquids: a process for recovery from paper-based waste. *J. Chem. Technol. Biotechnol.* 84:1818-1827
70. Lawoko M, Henriksson G, Gellerstedt G (2006) Characterisation of lignin-carbohydrate complexes (LCCs) of spruce wood (*Picea abies* L.) isolated with two methods. *Holzforschung* 60:156-161.
71. Åkerholm M, Salmén L (2001) Interactions between wood polymers studied by dynamic FT-IR spectroscopy. *Polymer* 42:963-969.
72. Stevanic J, Salmén L (2009) Orientation of the wood polymers in the cell wall of spruce wood fibres. *Holzforschung* 63:497-503.
73. Schwanninger M, Rodrigues J C, Pereira H, Hinterstoisser B (2004) Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vibrational Spectroscopy* 36:23-40.
74. Ebringerova A, Hromadkova Z, Hribalova V, Xuc C, Holmbom B, Sundberg A, Willför S (2008) Norway spruce galactoglucomannans exhibiting immunomodulating and radical-scavenging activities. *International Journal of Biological Macromolecules* 42:1-5.

75. Wu J, Zhang J, Zhang H, He J, Ren Q, Guo M (2004) Homogeneous Acetylation of Cellulose in a New Ionic Liquid. *Biomacromolecules* 5:266-268.
76. Dence C W, Lin S Y (1992) Eds.; In *Methods in lignin chemistry*; Springer-Verlag: Heidelberg Berlin. 578.