



Determination of molecular weight distributions in native and pretreated wood



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ABSTRACT

The analysis of native wood components by size-exclusion chromatography (SEC) is challenging. Isolation, derivatization and solubilization of wood polymers is required prior to the analysis. The present approach allowed the determination of molecular weight distributions of the carbohydrates and of lignin in native and processed woods, without preparative component isolation steps. For the first time a component selective SEC analysis of sawdust preparations was made possible by the combination of two selective derivatization methods, namely; ionic liquid assisted benzoylation of the carbohydrate fraction and acetobromination of the lignin in acetic acid media. These were optimized for wood samples. The developed method was thus used to examine changes in softwood samples after degradative mechanical and/or chemical treatments, such as ball milling, steam explosion, green liquor pulping, and chemical oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The methodology can also be applied to examine changes in molecular weight and lignin-carbohydrate linkages that occur during wood-based biorefinery operations, such as pretreatments, and enzymatic saccharification.

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1. Introduction

Wood and other lignocellulosic feedstocks are an abundant source of polymeric raw materials, amenable to modification into value-added chemical and biofuel applications (Ragauskas et al., 2006; Naik, Goud, Rout, & Dalai, 2010; Belgacem & Gandini, 2008). Their effective utilization requires a clear understanding of their polymeric structure and the implication of alteration caused by various pretreatment or isolation processes. It is also important to understand how these changes affect the subsequent utilization of the biomass and its individual components.

The molecular weight (MW) determination of lignocellulosic materials tends to be laborious and time consuming. Isolated preparations of wood components are typically needed for good solubility of samples and a selective analysis (Sjöholm, 2003; Hortling, Turunen, & Kokkonen, 2003). Overall, the literature demonstrates that the compositional and structural heterogeneity of the intact cell wall imposes significant limitations for analysis of the molecular weights directly from wood. The selective and controlled alteration of the cell wall components *in situ*, and the

application of two complementing solvent systems, may provide an effective route to the analysis of wood in its native form. Our aim here is to demonstrate that it is possible to use distinct dissolution/reaction chemistries on lignin and carbohydrates to produce soluble derivatives that represent the native components.

Ionic liquids (ILs) provide a new analytical tool in the field of cellulose molecular weight analysis (Hallac & Ragauskas, 2011). Recently IL's have been successfully applied for the size-exclusion chromatography (SEC) as the mobile phase for chromatographic analyses (Kuroda, Fukaya, & Ohno, 2013). ILs also have the unique capacity to be used as solvents for the analysis of cellulosic substrates when derivatization is needed to obtain readily soluble samples (El Seoud, Koschella, Fidale, Dorn, & Heinze, 2007).

Zoia, King, and Argyropoulos (2011) have introduced a novel derivatization method based on benzoylation of ball milled wood in 1-allyl-3-methylimidazolium chloride ([amim]Cl). Salanti, Zoia, Tolppa, and Orlandi (2012) have modified the procedure and added acetylation as a secondary step aimed toward the selective analysis of lignin. Ball milling was used in these studies to enhance the overall solubility of wood in IL, but there is evidence that the MW of wood components may be altered during such preparative treatment (Fujimoto, Matsumoto, Chang, & Meshitsuka, 2005; Howsmon & Marchessault, 1959). For this reason the ball milling may limit the usefulness of utilizing IL as tool for component

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selective MW analyses. Recent studies have concluded that the dissolution of non-milled wood sawdust in certain ILs proceeds via an extraction type mechanism where the cellulose is the primarily dissolved component (Leskinen, King, Kilpeläinen, & Argyropoulos, 2013; Casas, Alonso, Oliet, Santos, & Rodriguez, 2013). On this basis an effective method to directly dissolve cellulose for MW determination can be developed. The poor solubility of the lignin from non-milled wood and the uncertainties related to the use of ball milled wood for lignin MW analyses highlights the need for other solvent systems.

Instead of the derivatization in IL, an alternative approach for lignin acetylation is the acetobromination procedure that has been used to dissolve and analyse wood (Iiyama & Wallis, 1990). Recently this approach has been successfully applied for the analysis of isolated lignin preparations (Asikkala, Tamminen, & Argyropoulos, 2012). Both the acidic reaction media and the strongly acidic HBr by-product from the acetyl bromide, can degrade the carbohydrates in wood. At the same time, the native structure of lignin is retained and it becomes soluble with the introduction of acetyl and bromide functionalities.

The objective of this study was to create a facile methodology for the determination of molecular weight distribution (MWD) of native and pre-treated woods. To do this we have examined and optimized the analytical procedure and investigated the effects of preparative milling and possible side reactions known to occur during the various derivatization steps. The work, detailed below, has led to the development of an optimized protocol for such analyses.

2. Experimental

2.1. Materials

The solvents were purchased from Sigma-Aldrich and Fischer scientific and used without purification, except allyl chloride and 1-methylimidazole that were distilled prior to use. The fibrous cellulose powder was received from Whatman International Ltd. (grade CF1). enzymatic mild acidolysis lignin (EMAL) was prepared from ball-milled Norwegian Pine sawdust following the original procedure by Wu and Argyropoulos (2003). Enzymatic hydrolysis (48 h, 40 FPU of Viscozyme[®] enzyme cocktail from Novozymes, USA) was followed by mild hydrolysis in dioxane-water (85:15 v/v) containing 0.01 M HCl. The product was collected by precipitation from pH 2 HCl solution, and washed with deionized water.

Thermomechanical pulp (TMP) was obtained from a pulp mill located in Sweden. Material was extracted with acetone for 18 h, Wiley milled using 0.85 mm screen, and then a fraction below 0.40 mm size fraction was sieved off. Planetary ball milling of the resulting material was performed using a 250 ml zirconium bowl, loaded with eight zirconium balls (10 mm in diameter) and 2 g of TMP using a Microwolf planetary mill (Torrey Hills Technologies, USA) at 400 rpm. The milling sequences were set to 20 min milling and 20 min break times, totaling 1, 10 and 20 h. Samples of Norwegian Pine and Birch sawdust were received from BioOil AS (Norway), extracted with dichloromethane for 24 h, and sieved to particle size fractions of 0.85–0.25 mm and <0.25 mm. Bleached softwood pulp was received from a pulp mill located in the USA. Green liquor pulp from Pine (lignin content approx. 20 wt.%) was produced in-house according to the procedure described elsewhere (Wu, Chang, Jameel, & Philips, 2010). Hydrolysis was done in acetate buffer at 50 °C during 72 h in presence of 40 FPU of cellulolytic enzymes.

Synthesis of 1-allyl-3-methyl-imidazolium chloride ([amim]Cl) was performed as described elsewhere (Leskinen, King, Kilpeläinen, & Argyropoulos, 2011), with the exceptions that synthesis was carried out with 1.05 molar equiv. of 1-methylimidazole in relation to allyl chloride, and the charcoal purification was done using

acetonitrile as solvent during 24 h stirring at room temperature. Purity of the final product was confirmed by ¹H NMR.

2.2. Benzoylation

For the dissolution of wood prior to reaction, 1 g of ionic liquid was first weighed into a 8 ml screw cap vial with a magnetic stirring bar. Then 200 µl of 1-methylimidazole/pyridine 3:1 co-solvent mixture was added and the solvent was homogenized with vortex mixing. Vacuum-oven dried (24 h, RT) wood sawdust (particle size <0.25 mm) was added in 10 mg portion and mixed well using a vortex mixer. The sample was then placed in an oil bath at 60 °C (or 80 °C as specified in the text), and allowed to dissolve using stirring speed of 200 rpm. After an incubation time of 66 h (or specified times reported in the text), the viscous solution still containing some solids was taken out of the bath and cooled for a few minutes, and then 112 µl of benzoyl chloride was added. The reagent was mixed with the wood solution on the vortex mixer (~5 s), and then the sample was left to react for 4 h at room temperature using slow magnetic stirring. The reaction was stopped by the addition of 4 ml of 75% ethanol, and again mixed on the vortex mixer for 1 min. The precipitated mixture then was transferred into a centrifuge tube using the 75% ethanol solution and finally the solvent volume was adjusted to 20 ml. The washing solvent was removed using centrifuge, and the resulting solids were washed twice with 20 ml of ethanol by shaking and centrifuging the solvent. The solid product was left to dry under low vacuum overnight, and further dried in a room temperature vacuum oven. The experiments where benzoylation procedure was carried out without co-solvent, were done as described by in the original publication (Zoa et al., 2011). The dextran standards were benzoylated similarly as the wood samples, but the dissolution step was done at 40 °C temperature. The samples were fully dissolved after 66 h, and then benzoylated.

2.3. Acetobromination

The procedure from Asikkala et al. (2012) was optimized for this work. A dried 10 mg wood sample (particle size 0.85–0.25 mm) was weighed into a 8 ml screw cap vial and then dispersed to 2 ml of glacial acetic acid. The reaction was started by adding 218 µl of acetyl bromide. The sample was protected from light and left to stir at room temperature, maintaining 300 rpm magnetic mixing. Reaction time of 42 h was used for all samples, if not specified otherwise. After the specified reaction time, the dissolved sample was transferred to a 50 ml round bottom flask, and the solvent was evaporated using a high vacuum rotary evaporator. When the solvent had evaporated completely, the solid sample was dissolved in 30 ml of dimethylformamide (DMF) for the SEC analysis.

2.4. UV-spectroscopy

UV-absorption was measured from the same sample solutions of benzoylated and acetobrominated samples that were used for SEC analysis after 1/5 dilution with DMF. Exact concentrations of 0.5 mg/ml were used for benzoylated CF1 cellulose and lignin. Absorption spectra were recorded with a Beckman DU 640 spectrophotometer using quartz cuvettes.

2.5. Size-exclusion chromatography (SEC)

The samples were dissolved directly after derivatization and solvent removal in 20 ml (benzoylated) or 30 ml (acetobrominated) of DMF. Concentrations were empirically optimized based on the average soluble fractions of the samples and corresponding strength of the UV signal. Samples were allowed to dissolve for 1 h for benzoylated samples and 15 min for acetobrominated

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