

## Fractionation by sequential anti-solvent precipitation of grass, softwood and hardwood lignins isolated using low-cost ionic liquids and water

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6 Fractionation by sequential anti-solvent precipitation  
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10 of grass, softwood and hardwood lignins isolated  
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14 using low-cost ionic liquids and water  
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6 KEYWORDS  
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9 Lignin; ionic liquids; pretreatment; fractionation; sequential precipitation; anti-solvent;  
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13 molecular weight.  
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17  
18 ABSTRACT  
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23 In this study, fractionation by sequential anti-solvent precipitation was applied to IonoSolv  
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26 lignins for the first time. Pretreatment with the aqueous low-cost protic ionic liquid *N,N*-  
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29 dimethylbutylammonium hydrogen sulfate ([DMBA][HSO<sub>4</sub>], 80 wt% in water) was applied  
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33 to *Miscanthus* (herbaceous), willow (hardwood) and pine (softwood) to extract lignin.  
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36  
37 Then, lignin was sequentially precipitated by addition of water as anti-solvent.  
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41 Fractionation appeared to be controlled by molecular weight of lignin polymers. Fractions  
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44 isolated with minimal water volumes were shown to have high molecular weight,  
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46  
47 polydispersity, thermal stability and T<sub>g</sub> (178 °C). Later precipitates were more  
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50  
51 monodisperse, had high phenolic and total hydroxyl content, lower thermal stability and  
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53  
54 T<sub>g</sub> (136 °C). Addition of 1 g of water per g of dry IL was able to precipitate up to 90 wt%  
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3 of lignin. Fractional precipitation represents a novel lignin isolation technique that can be  
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5  
6 performed as part of the lignin recovery procedure enabling a high degree of control of  
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8  
9 lignin properties. The effect of the fractionation on lignin structural, chemical and thermal  
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11  
12 properties was thoroughly examined by 2D HSQC NMR, GPC, TGA and DSC and  
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14  
15 compared to the unfractionated lignin precipitate obtained by addition of an excess of  
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20 water.  
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## 25 INTRODUCTION

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28 Lignin valorization is critical for economic viability of biorefining processes, and is favored  
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31 by a low degree of condensation, chemically labile groups for functionalization, and high  
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34 purity<sup>1</sup>. Lignins with high molecular weight are suitable as fillers in composite materials<sup>2</sup>,  
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36  
37 while production of lignin-based adhesives replacing phenol requires low molecular mass  
38  
39  
40 and narrow weight distribution<sup>3</sup>. However, lignin is highly heterogeneous, with variations  
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42  
43 in its molecular weight, chemical composition, degree of cross-linking and functional  
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45  
46 groups, which makes its utilization challenging. Furthermore, lignin characteristics vary  
47  
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49  
50 depending on the biomass species it is isolated from and the extraction process used<sup>4</sup>.  
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3 Nearly all commercial scale cellulose-lignin separation occurs in paper mills using the  
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6 Kraft process, Kraft lignin in the form of “black liquor” is produced alongside Kraft pulp at  
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10 a volume of around 70 million tonnes per year<sup>5</sup>. However, less than 100 ktonnes are  
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13 available in isolated form<sup>6</sup> due to its structural complexity, high polydispersity and variable  
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16 functional group distribution. This has prevented most of the potential applications of  
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20 lignin explored in the literature from becoming commercially successful<sup>7</sup>.  
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23  
24 Fractionation of lignin has been widely investigated as a means of obtaining lignin  
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26  
27 fractions with narrow molecular weight distribution, well-defined chemical composition  
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29  
30 and functionality<sup>4,8</sup>. Four major lignin fractionation approaches have been reported: (1)  
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32  
33 selective precipitation by acid addition; (2) membrane-assisted ultrafiltration; (3) solvent  
34  
35  
36 extraction; and (4) fractional precipitation by sequential addition of anti-solvent. Acid  
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39 precipitation is the most common method, used in Kraft pulping<sup>9,10</sup>. Lignin is precipitated  
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42 by gradual acidification of the alkaline black liquor by addition of a strong acid, an easy  
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45 and low-cost process. However, the precipitated lignin presents high heterogeneity which  
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51 hinders its further valorization<sup>10</sup>. Hence, more selective methods, allowing better control  
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55 over molecular weight, have been proposed. Membrane technologies such as  
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3 ultrafiltration have been shown to allow efficient control of lignin molecular mass  
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6 distribution and to reduce contamination with hemicelluloses<sup>9,11</sup>. However, high  
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9 equipment cost, energy requirements and membrane fouling are major limitations<sup>12</sup>.  
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13 Successive extraction with different organic solvents can achieve remarkable fine-tuning  
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16 of lignin structural and chemical properties,<sup>13</sup> but these methods are largely based on  
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19 hazardous solvents, leading to high costs and safety risks<sup>2</sup>.  
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24 An alternative method is dissolving lignin in a pure or aqueous solvent, followed by  
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26  
27 fractional precipitation using an anti-solvent<sup>8</sup>. A fractional precipitation scheme for lignin  
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29  
30 was first proposed by Cui *et al.*<sup>14</sup>, based on partial solubility of lignin polymers in a  
31  
32  
33 solvent/anti-solvent mixture<sup>15</sup>. They demonstrated the use of a continuous solvent  
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36 gradient involving two miscible organic solvents, one being a good solvent for Kraft lignin  
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39 (acetone) and the other poor (hexane). A relatively monodisperse fraction with Mw of  
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41  
42 1500 and polydispersity approaching 1.1 was successfully isolated in yields ranging  
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44  
45 between 10 and 20% w/w<sup>14</sup>. A similar scheme, using water instead of hexane, was  
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48 adopted by Sadeghifar *et al.*<sup>4</sup> for Organosolv lignin. Wang et al. employed  $\gamma$ -valerolactone  
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3 as solvent for lignin fractionation when mixed with water and used Kamlet-Taft  
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6 parameters to study the fractionation mechanism<sup>16</sup>.  
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10 This study is the first application of sequential precipitation to lignins from herbaceous,  
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12  
13 hardwood and softwood biomass isolated using the “ionoSolv” pretreatment process,  
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16 developed by Hallett *et al.*<sup>17–19</sup> It uses low-cost amine-based aqueous protic ionic liquids  
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18  
19 (ILs) to dissolve and recover high-purity lignins<sup>20</sup>. Water was chosen as anti-solvent due  
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21  
22 to its low cost, high hydrogen bonding capability, non-hazardous nature and efficient  
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25 precipitation ability<sup>20</sup>. This represents a novel lignin fractionation technique for lignin  
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27  
28 recovery. Finally, the structural and chemical properties of lignin fractions were thoroughly  
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30  
31 characterized.  
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## 36 37 38 39 40 MATERIALS AND METHODS

### 41 42 43 **Materials**

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47 *Miscanthus x giganteus* was obtained from Imperial College London Silwood Park  
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50 campus (Berks, UK) in April 2016. Willow (*Salix redheriana* ‘Endurance’ variety, Plot 22  
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52  
53 Trial C8689) was obtained from Rothamsted Research Ltd (Harpenden, UK) in January  
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3 2014. Virgin pine (*Pinus sylvestris*) was sourced from Metla (Finish Forest Research  
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5  
6 Institute) in 2010. All feedstocks were air-dried at room temperature for 72 h. They were  
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8  
9 then ground (Retsch AS200 cutting mill, 1 cm square mesh) and sieved (0.18-0.85 mm,  
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11  
12 US mesh scale -20/+80) and stored air-dry in sealed plastic bags at ambient temperature.  
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15  
16 IL moisture content was measured with a Karl-Fischer V20 volumetric Titrator (Mettler-  
17  
18  
19 Toledo). Acid:base ratios were determined with a Mettler Toledo G20S compact titrator.  
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21  
22  
23 pH measurements were performed using a Jenway 3510 equipped with VWR Ag/AgCl  
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25  
26 symphony electrode. Weight measurements were performed on a Sartorius CPA 1003S  
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30 analytical balance ( $\pm 0.001$  g).  
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### 33 34 **Ionic Liquid Synthesis**

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37 All pretreatment experiments in this study used a 80 wt% aqueous solution of *N,N*-  
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40 dimethylbutylammonium hydrogen sulfate ([DMBA][HSO<sub>4</sub>]), a protic IL synthesized by  
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42  
43 mixing H<sub>2</sub>SO<sub>4</sub> and *N,N*-dimethylbutylamine according to published procedures<sup>18</sup>, as  
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45  
46 pretreatment solvent. Two mixtures were prepared with acid:base molar ratios (a:b) of 1:1  
47  
48  
49 ([DMBA][HSO<sub>4</sub>]<sub>80%</sub> a:b=1:1) and 1.03:1 ([DMBA][HSO<sub>4</sub>]<sub>80%</sub> a:b=1.03:1) respectively. They  
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55 were characterized by <sup>1</sup>H NMR, <sup>13</sup>C NMR and LR-MS. Water content was measured by  
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3 Karl-Fischer titration. Titration against a solution of NaOH and pH measurements were  
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5  
6 made to verify the acid:base molar ratios. Full details can be found in the Supplementary  
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8  
9 Information.

### 13 **Compositional Analysis**

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15  
16 Compositional analysis was carried out in triplicate on untreated extractives-free  
17  
18 biomass feedstocks according to the published procedure Determination of Structural  
19  
20 Carbohydrates and Lignin in Biomass by the NREL<sup>21</sup>. Prior to undertaking compositional  
21  
22  
23 analysis, the extractives were removed from untreated biomass using ethanol following  
24  
25  
26 NREL-TP-510-42619<sup>22</sup>. Moisture content of untreated material was determined following  
27  
28  
29 NREL/TP-510-42618<sup>21</sup>. Further details can be found in the Supplementary Information.  
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37 The moisture contents of untreated *Miscanthus*, willow and pine were 6.8%, 5.2% and  
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40  
41 6.7%, respectively.

### 44 **Ionic Liquid Pretreatment**

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47 Before pretreatment, the water content of [DMBA][HSO<sub>4</sub>] was adjusted to 20 wt%  
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49  
50 considering the moisture content of the untreated biomass. The three feedstocks -  
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54  
55 *Miscanthus*, willow and pine- were separately pretreated according to a scaled-up  
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3 pretreatment procedure, based on the 10 mL-scale procedure published previously<sup>18</sup>.  
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6 Pretreatments were carried out in a 1.5-L jacketed borosilicate glass pressure vessel with  
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9 stirring (Büchi AG, Switzerland). Full details of the reactor setup and adapted  
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12 pretreatment protocol can be found in the Supplementary Information, including the  
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16 cooling conditions and a sample cooling ramp. For safety purposes, the reactor vessel  
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19 was equipped with a proportional relief valve set to 4.5 barg and a bursting disk (6 barg,  
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22 with lowest rating of 5 barg at 150 °C). The maximum recorded pressure at 150 °C was  
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25  
26 3.70 bara. Reactions were carried out in monoplicate at 150 °C, using a process volume  
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29 of 1 L. Stirring was performed using a 50 mm anchor agitator with flat blades set at  
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31  
32 constant stirring rate of 100 rpm. A biomass to solvent ratio of 1:5 g/g was used  
33  
34  
35 (equivalent to 20 wt% loading) with 1000 g of 80 wt% [DMBA][HSO<sub>4</sub>] and 200 g of  
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37  
38 biomass on a dry basis in all experiments. *Miscanthus*, willow and pine were pretreated  
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40  
41 for 120, 90 and 90 minutes, respectively. The pretreatment conditions are summarized in  
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44  
45 the Supplementary Information (Table S1).  
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51 After cooling of the reaction mixture, the vessel contents were unloaded by washing with 1 L of  
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53 absolute ethanol. The IL-biomass-ethanol slurry was transferred to four 500 mL centrifuge tubes  
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55 (Corning, USA), allowed to equilibrate for 1 h and centrifuged (Megastar 3.0, VWR, UK; 3000  
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59  
60

rpm, 50 min). The liquid phase was decanted and collected, while the pulp was washed 14 more times with ethanol. Recovered IL and ethanol washes were combined, and ethanol was removed by rotary evaporation (by heating at 40 °C under reduced pressure) until complete ethanol removal was achieved. The recovered IL liquor (containing dissolved lignin) was collected and stored in a 1 L sealed glass bottle for a maximum period of 4 weeks before lignin precipitation was carried out.

### Lignin Fractionation by Sequential Precipitation

Water content of the liquor was measured by Karl-Fischer titration in triplicate (typically it was found to be ~5–10 wt%). Lignin was fractionally precipitated by sequential addition of anti-solvent (deionized water). Each liquor was split into six different fractions: three fractions of ~50–75 g liquor each were used for “full precipitation” of lignin, while three batches of ~250 g liquor each were used for “fractional precipitation”. The amount of water needed was calculated by weight “equivalents” (eq) of water compared to the amount of dry IL in the liquor. Equations 1 and 2 were used to calculate the equivalents of water in a given liquor sample:

$$\text{Equivalents} = \frac{m_{\text{water},f}}{m_{\text{dry IL}}} = \frac{m_{\text{liquor},f} \times \text{WC}_{\text{liquor},f}}{m_{\text{liquor},f} \times (1 - \text{WC}_{\text{liquor},f})} \quad \text{Equation 1}$$

$$\text{where} \quad m_{\text{water},f} + m_{\text{dry IL}} = m_{\text{liquor},f} \quad \text{Equation 2}$$

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5 where  $m_{water,f}$  is the final amount of water in the liquor (g),  $m_{dry,IL}$  is the weight of dry IL in  
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7  
8 the liquor (g),  $m_{liquor,f}$  is the final mass of liquor sample (g) and  $WC_{liquor,f}$  is the final liquor  
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11  
12 water content as determined by Karl-Fischer titration (wt/wt).  
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15 The amount of water  $m_{water\ added}$  to be added to a given IL liquor, in order to attain a  
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18 specific final water content  $WC_{liquor,f}$  was calculated using Equations 3 and 4:  
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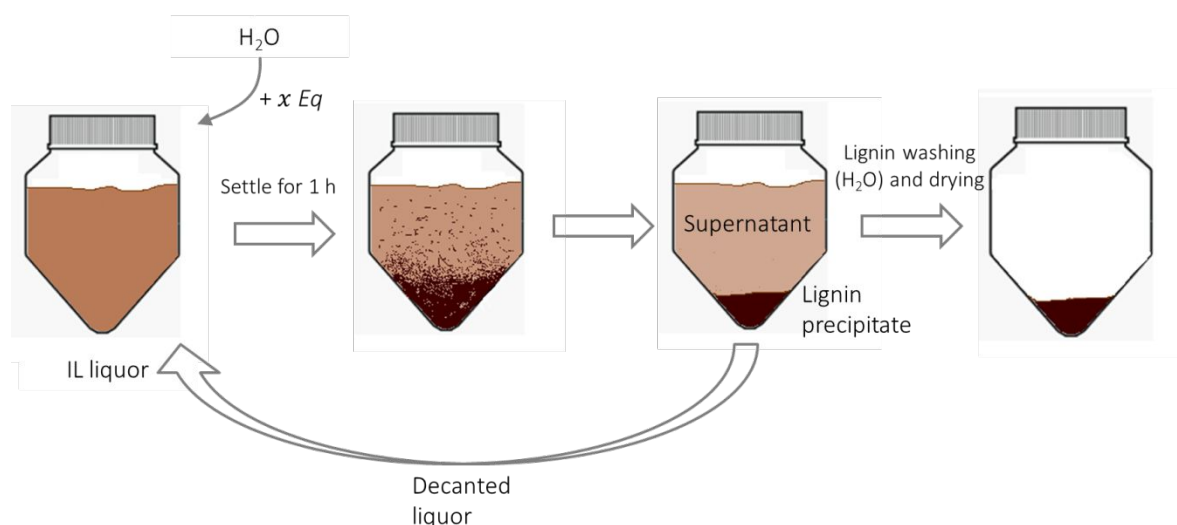
$$m_{water\ added} = Eq \times [m_{liquor,i} \times (1 - WC_{liquor,i})] - m_{liquor,i} \times WC_{liquor,i} \quad \text{Equation 3}$$

$$\text{where} \quad WC_{liquor,f} = \frac{m_{water\ added} + m_{liquor,i} \times WC_{liquor,i}}{m_{water\ added} + m_{liquor,i}} \quad \text{Equation 4}$$

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35 where  $m_{liquor,i}$  (g) is the total weight of the initial liquor,  $WC_{liquor,i}$  (wt/wt) its initial water  
36  
37  
38 content,  $m_{water\ added}$  (g) is the amount of water needed to precipitate the next lignin fraction,  
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40  
41  
42  $m_{liquor,f}$  is the final mass of liquor (g) after adding the water and  $WC_{liquor,f}$  is the final liquor  
43  
44  
45 water content as determined by Karl-Fischer titration (wt/wt) after addition of water.  
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49 Full lignin precipitation was carried out by addition of 5 eq of water, as calculated using  
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51  
52 Equations 3–4, to a batch of ~50–75 g of liquor weighed into a 500 mL centrifuge tube  
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(Corning, USA), in a single step. We are here considering “full” precipitation to be defined as the amount of lignin recovered from the addition of 5 equivalents of water to the liquor, beyond which very little lignin can be precipitated. It is not the entirety of the dissolved lignin, as small, water-soluble fragments are expected to remain in the liquor. The solution was allowed to equilibrate overnight and the precipitated lignin was separated by centrifugation (3500 rpm, 120 min). The supernatant was carefully decanted and discarded. Residual IL was removed by washing the lignin with 500 mL of deionized water, allowing to equilibrate for 1 h and centrifuging as above. This was repeated 4 times. Then, it was freeze-dried (Labconco Freezone 6, 0.016 mbar, -54 °C) for 48 h, and weighed. Full lignin precipitation was carried out in triplicate for each of the four sets of experiments performed.



**Scheme 1.** Schematic representation of the lignin fractionation process by which lignins were precipitated and recovered by sequential anti-solvent addition.

Fractional lignin precipitation was carried out by sequential addition of 0.25, 0.5, 1, 1.5, 2, 2.5 and 5 equivalents of water. Up to 7 precipitates per initial liquor sample were recovered. For this, ~250 g of liquor was weighted into a 500 mL centrifuge tube and, for each step, the desired amount of water, calculated using Equations 3–4, was added. The solution was allowed to equilibrate for 1 h and the precipitated lignin was separated by centrifugation (3500 rpm, 120 min). The supernatant was carefully decanted into a clean pre-weighed 500 mL centrifuge tube and the weight of liquor was recorded. The solid precipitate was washed four times with deionized water. The supernatant was transferred as before and the next precipitation step was performed. The whole process is shown diagrammatically in Scheme 1. Fractional precipitation was carried out in triplicates using three batches of ~250 g of the original liquor recovered from each pretreatment experiment for each of the four sets of experiments performed.

### Lignin Mass Balance

The lignin mass balance was determined by weighing each sample obtained from precipitation. Then, the lignin weight for each sample,  $m_{lignin,x}$ , was normalized to the amount of dried IL in the initial liquor,  $m_{liquor,i,x}$  in each precipitation step, as shown in Equations 5 and 6:

$$Yield (x Eq) = \frac{m_{lignin,x}}{m_{dry IL,x}} \times 100 \quad \text{Equation 5}$$

where  $m_{dry IL,x} = m_{liquor,i,x} \times (1 - wc_{liquor,i,x})$  Equation 6

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3 where  $m_{\text{lignin},x}$  is the mass of lignin (g) recovered after addition of  $x$  equivalents of water in total to  
4  
5 a given liquor with initial liquor mass  $m_{\text{liquor},I,x}$  (g) and water content  $wc_{\text{liquor},I,x}$  (wt/wt).  
6

7 The total amount of lignin obtained by full precipitation was calculated for each of the triplicates  
8  
9 and the mean value reported in units of g lignin per 100 g of dry IL liquor. The total amount of  
10  
11 lignin precipitated by fractional precipitation was calculated as the average sum of all the lignin  
12  
13 yields obtained from Equation 5 for a given fractional precipitation experiment.  
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### 16 **Gel Permeation Chromatography**

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18 GPC measurements were performed using an Agilent 1260 Infinity instrument equipped with a  
19  
20 Viscotek column set (AGuard, A6000M and A3000M). An Agilent 1260 Infinity RID detector  
21  
22 was used for detection. HPLC grade DMSO containing LiBr (1 g/L) was used as eluent at a flow  
23  
24 rate of 0.4 mL/min at 60 °C. Samples were prepared by dissolving 20 mg lignin in 1 mL eluent  
25  
26 and filtering through a 0.25  $\mu\text{m}$  syringe filter. Ten pullulan standards (Agilent calibration kit, 180  
27  
28  $< M_p < 780,000$ ) were used to calibrate the instrument.  
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### 32 **2D HSQC NMR**

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34  $^{13}\text{C}$ - $^1\text{H}$  heteronuclear single quantum coherence (HSQC) NMR spectroscopy was performed for  
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36 selected lignin fractions (the first two precipitates from sequential precipitation, the last precipitate  
37  
38 eq = 5, and the full precipitate) from *Miscanthus* isolated after 120 min at 150 °C using  
39  
40 [DMBA][HSO<sub>4</sub>]<sub>80%</sub> a:b=1:1 and [DMBA][HSO<sub>4</sub>]<sub>80%</sub> a:b=1.03:1. Samples were prepared by  
41  
42 dissolving ca. 20 mg of lignin in 0.25 mL of DMSO- $d_6$  in a Shigemi tube. HSQC NMRs were  
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44 recorded on a Bruker 600 MHz spectrometer (pulse sequence hsqcetgpsi2, spectral width of 10  
45  
46 ppm in F2 ( $^1\text{H}$ ) with 2048 data points and 160 ppm in F1 ( $^{13}\text{C}$ ) with 256 data points, 16 scans and  
47  
48 1 s interscan delay). The spectra were referenced to the DMSO solvent peak at  $\delta\text{H}/\delta\text{C}$   
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55 2.50/39.5 ppm. Spectral assignment was conducted according to our previous works for  
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3 grass<sup>19</sup>, hardwood<sup>23</sup> and softwood<sup>24</sup>, respectively. Signal intensities were normalized to  
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6 the total abundance of  $G_2 + G_{2,cond}$  signals. The total ( $G_2 + G_{2,cond}$ ) and ( $S_2 + S_{2,cond}$ ) were  
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8  
9 used to calculate the S/G ratio, after halving the contribution from S units to avoid double-  
10  
11  
12 counting for the symmetrical syringyl unit. The degree of condensation was estimated as  
13  
14  
15  
16 the proportion of  $G_2$  units involved in condensation reactions, i.e.  $G_{2,cond} : (G_2 + G_{2,cond})$ .  
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18  
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### 20 <sup>31</sup>P NMR Spectroscopy

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22  
23 Quantitative <sup>31</sup>P NMR spectra of all lignin preparations were obtained using published  
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25  
26 procedures<sup>25</sup>. The methodology is reported in the Supplementary Information. Vortex  
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29 mixing of the prepared samples was carried out for at least 20 min or until the sample as  
30  
31  
32 fully dissolved. <sup>31</sup>P NMR experiments were recorded for selected lignin fractions (the first  
33  
34  
35 two precipitates from sequential precipitation, the last precipitate eq = 5, and the full  
36  
37  
38 precipitate) from *Miscanthus* isolated after 120 min at 150 °C using [DMBA][HSO<sub>4</sub>]<sub>80%</sub>  
39  
40  
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44  
45 a:b=1:1 and [DMBA][HSO<sub>4</sub>]<sub>80%</sub> a:b=1.03:1.  
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47

### 48 Elemental Analysis

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50  
51 CHN elemental analyses of the lignins was carried out in a Vario MICRO Cube analyzer  
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53  
54  
55 (Elementar, Germany) using 1-2 mg of the sample, and helium as the carrier and flushing  
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3 gas. Each sample was analyzed three times and the average was taken. Residual IL  
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6 contamination was calculated using the nitrogen and sulphur content to calculate the  
7  
8  
9 cation and anion contributions, respectively. The sum of the two was used to obtain the  
10  
11  
12 residual IL content in the lignins. The oxygen content in the samples was estimated by  
13  
14  
15 difference. Accuracy is  $\pm 0.20\%$  absolute. The higher heating values were calculated  
16  
17  
18 according to a correlation developed by Demirbas<sup>26</sup>:  
19  
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21

$$22 \quad \text{HHV} = (33.5[C] + 142.3[H] - 15.4[O] - 14.5[N]) \quad \text{Equation 7}$$

23  
24 where  $C$  = carbon content,  $H$  = hydrogen content,  $O$  = oxygen content, and  $N$  = nitrogen  
25  
26  
27 content. In this study, the elemental compositions of the raw materials were used as  
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29  
30  
31  
32 received. HHV were calculated from elemental analysis values obtained in triplicates.  
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35

### 36 **Thermogravimetric Analysis**

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40 Thermogravimetric analysis was conducted on a TA Q500 (TA Instruments, USA) TGA  
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42  
43 analyzer fitted with a gas switching system, using a platinum pan, with  $N_2$  as the purge  
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45  
46 gas (50 mL/min).  $3 \pm 2$  mg of previously freeze-dried lignin was loaded per run; each  
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48  
49  
50 sample was analyzed in duplicate. For proximate analysis of sample composition, a  
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54 drying step at 105 °C for 15 min was carried out before linearly ramping the temperature  
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3 at 60 °C/min over a temperature window of 105 to 600 °C. The sample was held at 600  
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5  
6 °C for 15 min before a burn-off step was initiated by switching the purge gas to 10% air  
7  
8  
9 and 90% nitrogen to remove all organic material and determine the ash content. The  
10  
11  
12  
13 sample weight after 15 min (dry weight), 39 min (fixed carbon and ash weight) and 54 min  
14  
15  
16 (ash weight) were determined and fixed carbon content and ash content were calculated  
17  
18  
19 using Equations 8 and 9, respectively, where  $m_t$  is the weight fraction of the sample at a  
20  
21  
22  
23  
24 given time  $t$  expressed in minutes:  
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26  
27

$$\text{Fixed carbon content} = \frac{m_{39 \text{ min}} - m_{54 \text{ min}}}{m_{15 \text{ min}}} \cdot 100\% \quad \text{Equation 8}$$

$$\text{Ash content} = \frac{m_{54 \text{ min}}}{m_{15 \text{ min}}} \cdot 100\% \quad \text{Equation 9}$$

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36  
37 The proximate analysis data can be found in the Supplementary Information (Table  
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39  
40  
41 S11).  
42  
43

44  
45 For thermal analysis, the TGA weight loss curves and their first derivatives, i.e. the  
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47  
48 differential thermogravimetric (DTG) curves with respect to temperature for all lignins  
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50  
51 were plotted and used to identify the temperature at which the DTG curve showed the  
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55 maximum rate of thermal composition ( $\text{DTG}_{\text{max}}$ ).  
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## Differential Scanning Calorimetry

DSC measurements of lignin samples were performed, in duplicates, on a DSC 2500 calorimeter (TA Instruments, USA). The analyses were conducted using  $5.5 \pm 0.5$  mg of lignin in hermetically sealed aluminium pans (Tzero pan and hermetic lid;  $40 \mu\text{L}$ ). In order to eliminate the thermal history of the samples, an initial heating, annealing and cooling cycle was carried out to clear their stored thermal history within the polymer's glassy state. To overcome the strong enthalpy relaxation of amorphous lignin samples for the purposes of an accurate determination of the glass transition temperature ( $T_g$ ), an annealing step above the lignin  $T_g$  was added using a heat-cool-heat experiment. The samples were subjected to an initial scan where they were heated from  $20^\circ\text{C}$  to  $160^\circ\text{C}$  with a heating rate of  $10^\circ\text{C}/\text{min}$  and maintained at  $160^\circ\text{C}$  for 10 min. They were then cooled down to  $-20^\circ\text{C}$  with a cooling rate of  $10^\circ\text{C}/\text{min}$  and maintained at this temperature for 10 min. A second heating run was used to determine the glass transition temperatures ( $T_g$ ), by ramping the temperature from  $-20^\circ\text{C}$  to  $200^\circ\text{C}$  at  $20^\circ\text{C}/\text{min}$  (as the glass transition is more pronounced at faster heating rates), followed by an isothermal state at  $200^\circ\text{C}$  for 10 min and cooling to room temperature at  $30^\circ\text{C}/\text{min}$ . All DSC scans were conducted

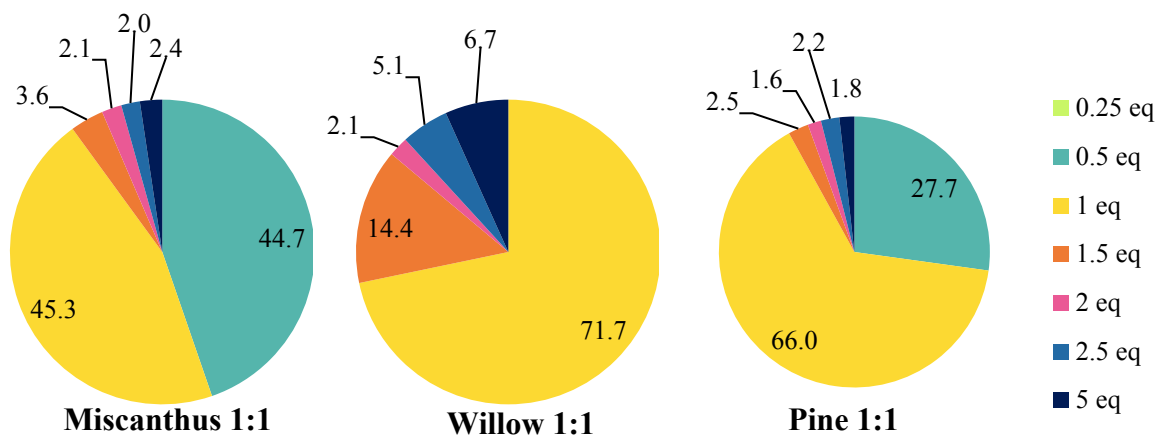
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3 under air with a flow rate of 50 mL/min. DSC analysis was performed using TA  
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6 Instruments TRIOS software to determine the  $T_g$  using a double tangent method.  
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## 9 10 RESULTS & DISCUSSION

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13 Lignin fractionation by sequential anti-solvent precipitation was investigated for  
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16 ionoSolv lignins for the first time. *Miscanthus*, willow and pine, which are classified,  
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19 respectively, as herbaceous biomass, hardwood and softwood feedstocks, were  
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23 pretreated using the low-cost protic IL [DMBA][HSO<sub>4</sub>] containing 20 wt% water at 150 °C,  
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27 for residence times between 90 and 120 min, under agitation, using a biomass to solvent  
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31 ratio of 1:5 g/g. The pretreatment conditions were selected according the maximum lignin  
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34 precipitate yields obtained on previous time course studies using protic ILs<sup>19,23,24,27-29</sup>.  
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38 Following pretreatment, the lignin dissolved in the IL-water mixture was sequentially  
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41 precipitated by addition of different volumes of water. The results of the lignin mass  
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44 balance and trends in the molecular weight, chemical functionality and thermal properties  
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47 of lignin fractions are presented.  
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### 50 51 **Mass Balance of Lignin Fractions**

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**Figure 1.** Mass balance of lignins isolated by sequential precipitation for *Miscanthus*, willow and pine. Lignin was extracted into [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1.

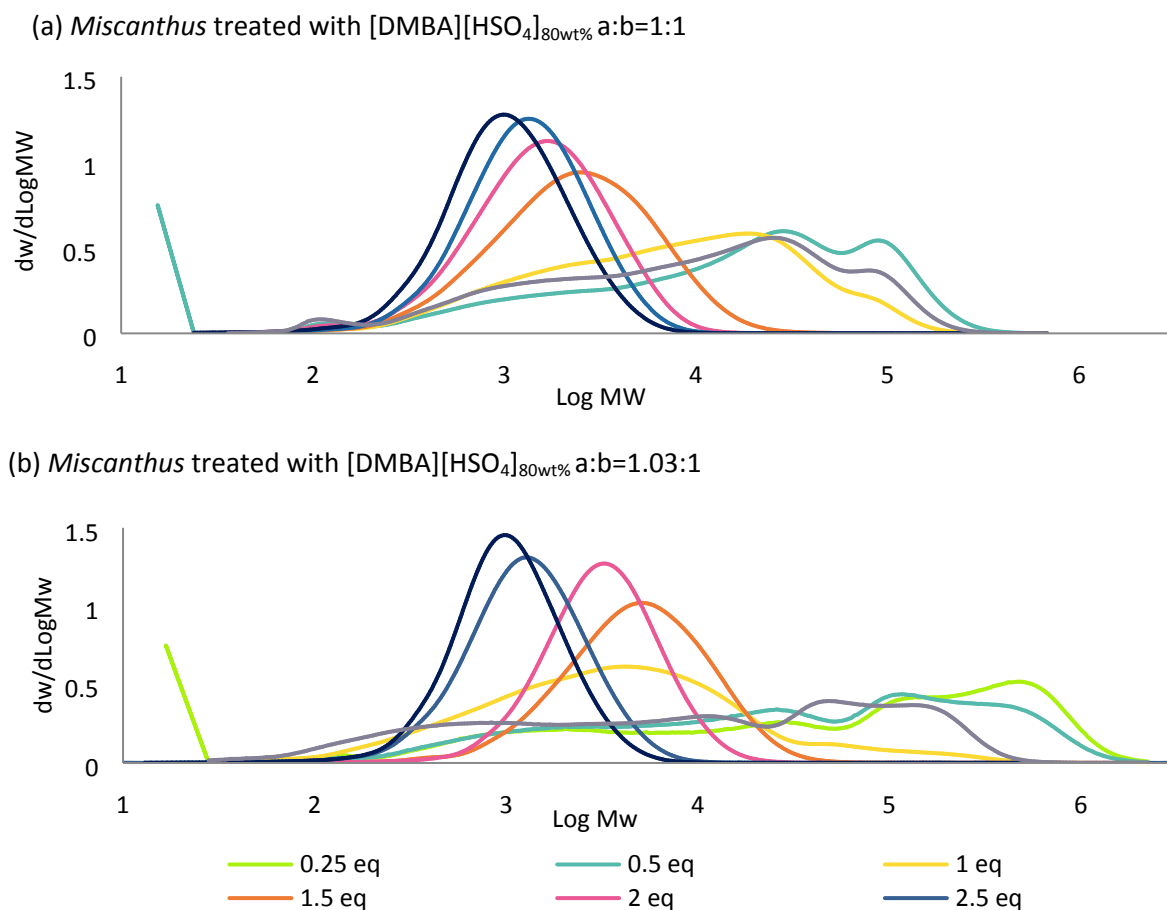
Figure 1 shows the mass balance of the precipitated lignin from 0.25 up to 5 eq of water (relative to original IL weight in solvent) for *Miscanthus*, willow and pine pretreated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1. Under these conditions, no lignin was precipitated after addition of 0.25 eq water. For *Miscanthus* and pine, lignin precipitation started after the addition of 0.5 eq of water; and over 90 wt% of lignin precipitated after the addition of 1 eq of water, compared to only 72% for willow lignin. This indicates that *Miscanthus* and pine lignin exhibit higher molecular weight or are more hydrophobic than willow lignin. The high content of S units in willow lignin might result in less condensed, less hydrophobic structures, as these are sterically hindered and, therefore, less reactive.<sup>30</sup>

## Molecular Weight Characterization by GPC

The molecular weight distributions of fractional and full lignin precipitates were examined by gel permeation chromatography. The eluted molecular weight profiles for *Miscanthus* lignins isolated using [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1 are shown in Figure 2, while the molecular weight profiles for willow and pine can be found in the Supplementary Information (Figure S2). Key molecular markers for lignins, including the weight-average molecular weights  $M_w$ , number-average molecular weight  $M_n$ , and the polydispersity index ( $PDI = M_w/M_n$ ) are displayed in Tables 1–2 for *Miscanthus* and in the Supplementary Information Tables S2 and S3, respectively, for willow and pine.

GPC findings confirmed that lignin fractionation was predominantly influenced by lignin molecular weight. The GPC profiles exhibited a marked shift towards lower molecular mass material with increasing anti-solvent addition, in agreement with previous reports from organic solvent and fractional precipitation studies<sup>4,13–15</sup>. Lignin with higher molecular weights were obtained after the addition of 0.25 to 1 eq of water, with a large proportion of material having molar mass  $> 30000 \text{ g mol}^{-1}$ . Nevertheless, small molecular

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3 mass species also precipitated together with the largest molecules, producing fractions  
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6 with high polydispersity  $> 10$ . In contrast, later fractions, obtained after the addition of  $> 1$   
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9 eq of water showed narrow molecular profiles, with peak molecular mass occurring  
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12 around  $1000 - 3000 \text{ g mol}^{-1}$ . For *Miscanthus*, full precipitate profiles, shown in grey, were  
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15 very polydisperse and most closely resembled GPC profiles of the early precipitates and  
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18 were intermediate between the precipitates for 0.5 and 1 eq for  $[\text{DMBA}][\text{HSO}_4]_{80\text{wt}\%}$   
19  
20  
21  $a:b=1:1$ , and 0.25 and 0.5 eq for  $[\text{DMBA}][\text{HSO}_4]_{80\text{wt}\%}$   $a:b=1.03:1$ . This is consistent with  
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27 the fact that these fractions combined made up  $\sim 90\%$  of species by weight. On the other  
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31 hand, for willow and pine the full precipitate was closest to the 1 eq fraction, which made  
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60 up 72 wt% and 66 wt% respectively.



**Figure 2.** GPC Chromatograms of full precipitation and sequential fractionated lignin as a function of water amount added for *Miscanthus* lignins with (a) [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and (b) [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.

**Table 1.** Yields and molecular markers for sequential and full precipitates isolated from *Miscanthus* pre-treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1.

Lignin Fractions	Yields (wt%)	M <sub>n</sub> (g/mol)	M <sub>w</sub> (g/mol)	PDI
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<b>0.5 equivalents</b>	44.7±1.9	2190	39700	18.1
<b>1 equivalent</b>	45.3±2.9	2080	18200	8.7
<b>1.5 equivalents</b>	3.6±0.6	1190	3400	2.8
<b>2 equivalents</b>	2.1±0.8	900	2000	2.2
<b>2.5 equivalents</b>	2.0±0.4	860	1600	1.9
<b>5 equivalents</b>	2.4±0.7	720	1300	1.8
<b>Full Precipitation</b>	100	1590	26300	16.6

<sup>a</sup> Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

Table 1 shows the percentage mass recovered and the Mw, Mn and PDI found with GPC of *Miscanthus* lignin treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1, both after sequential water addition and full precipitation. After full precipitation, *Miscanthus* lignin had a Mw of 26300 Da and a PDI of 16.6. After sequential precipitation, the PDI was higher for the first fraction (0.5 eq) where the Mw reached 39700 Da and the PDI 18.1. Mn, Mw and PDI all decreased over the following water additions; the bigger differences occurred between 1 and 0.5 eq, with a decrease to less than half for both the Mw and PDI. The lowest PDI (1.8) was found for the fraction precipitated after the addition of 5 eq of water.

Using the sequential water addition, almost half of the lignin (45 wt%, 1 eq water) was recovered at a substantially lower Mw and PDI than using the full precipitation. This is

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3 promising for potential commercial applications. Considering the GP chromatogram and  
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6 the smaller proportion of high Mw lignin in the 1 eq fraction, a narrower molecular weight  
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9 distribution may be achievable without compromising too much on yield by tuning the first  
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13 water addition (e.g. adding 0.6 equivalents instead of 0.5).  
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17 The molecular markers for lignins isolated from *Miscanthus* pre-treated with  
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20 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1 are shown in Table 2. Under these conditions, a  
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23 precipitate was recovered after the addition of 0.25 eq of water (64 wt%). This lignin had  
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26 extremely high molecular weight, with Mw of 207000 and polydispersity of 70.5. Later  
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29 fractions obtained with  $\geq 1.5$  eq of water strongly resembled their analogues from  
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33 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.1, with similar low molecular weight ( $M_n < 1,000$ ) and  
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36 polydispersity (1.8–3.1). The total yield of lignin was also higher. 10.2 g/100 g dry IL  
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39 (195% of native lignin) was obtained, compared to 7.6 g/100g dry IL (137% yield) for  
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44 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1. This can be explained by the increased propensity of lignin  
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47 to suffer condensation reactions under higher acid concentration, forming higher  
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51 molecular weighted lignins that are easily precipitated, increasing the lignin yield<sup>28</sup>. This  
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55 suggests the possibility of optimizing the energy intensity and product value of the  
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ionoSolv process by selection of IL acidity, allowing reduced reaction times and easily precipitated lignins with low water input.

**Table 2.** Yields and molecular markers for sequential and full precipitates isolated from *Miscanthus* pre-treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.

Lignin Fractions	Yields (wt%)	M <sub>n</sub> (g/mol)	M <sub>w</sub> (g/mol)	PDI
<b>0.25 equivalents</b>	64.1±2.7	2940	207000	70.5
<b>0.5 equivalents</b>	24.0±0.3	2710	151800	56.1
<b>1 equivalent</b>	6.9±0.1	2170	32100	14.8
<b>1.5 equivalents</b>	2.5±0.2	1250	3300	2.7
<b>2 equivalents</b>	0.9±0.2	1010	1900	1.9
<b>2.5 equivalents</b>	0.5±0.2	890	2700	3.1
<b>5 equivalents</b>	1.1±0.6	760	1400	1.8
<b>Full Precipitation</b>	100	2470	163800	66.2

<sup>a</sup> Mass recovery shown as a proportion of the total lignin obtained from fractional precipitation and for full precipitation.

For both IL compositions, increasing anti-solvent addition led to lower molecular weight lignin oligomers (~700–1000 g mol<sup>-1</sup>) and narrower molecular weight distribution (~2–3).

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3 Lignins with these desirable properties accounted for 5–10% of lignin mass. These  
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6 findings suggest fractionation could be a worthwhile technique to separate valuable  
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9 lignins from polydisperse macromolecules. Further optimisation, for instance by reducing  
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12 process intensity, could be used to increase yields of desirably monodisperse lignins.  
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### 16 **Structural Properties by HSQC NMR**

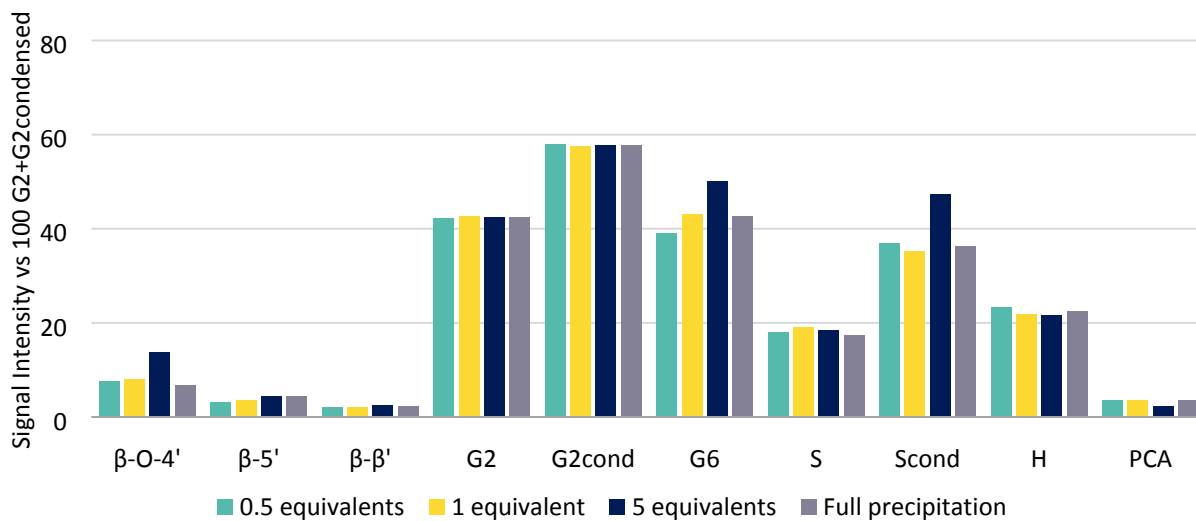
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20 The chemical structure of lignin fractions was investigated by 2D HSQC NMR, focusing  
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23 on the ether linkage content and the degree of C–C bond condensation. Figure 3 shows  
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25  
26 the signal intensities from HSQC NMR analysis of the first two lignin fractions (0.5 and 1  
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29 eq for [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1; 0.25 and 0.5 eq for [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1),  
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32 last lignin fraction (5 eq) and full precipitate from *Miscanthus* treatment with both ILs  
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35 (equivalent findings for willow and pine lignins and the spectra for all biomass types are  
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38 available in the Supplementary Information, shown in Figures S8 – S12). As more  
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41 equivalents of water were added, lignin fractions exhibited greater abundance of interunit  
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44 linkages β-*O*-4', β-5' and β-β' (Figure 4). Initial fractions had between one-third and half  
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47 of the ether linkage content compared to the final fraction. Ether bond content in lignin  
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51 fractions treated with excess acid was reduced due to known acid-catalyzed bond  
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3 cleavage<sup>31</sup>. Moreover, the intensity of the G<sub>2</sub> and G<sub>6</sub> signals were higher in the fractions  
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6 having lower molecular mass, which is consistent with a lower degree of C–C  
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9 condensation. As anticipated, the degree of condensation of lignin influenced its  
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12 molecular weight and, in turn, its solubility in water. Fractionation also influenced the p-  
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15 coumaric acid (PCA, Figure 4) signal intensity, which decreased in the later lignin  
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18 precipitates. Comparing the intensities of the signals from fractionated lignins and full  
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21 precipitates, it was observed that the signal intensities of full precipitates resembled those  
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24 of 1 eq for [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and 0.25 eq for [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1 as  
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27 these represent the bulk of lignin species. In the case of pine treated with  
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30 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1, the full precipitate resembled that of 0.5 eq (Figure S8). On  
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33 the other hand, for willow lignin the full precipitate appeared less condensed than  
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36 fractional precipitates (Figure S8).  
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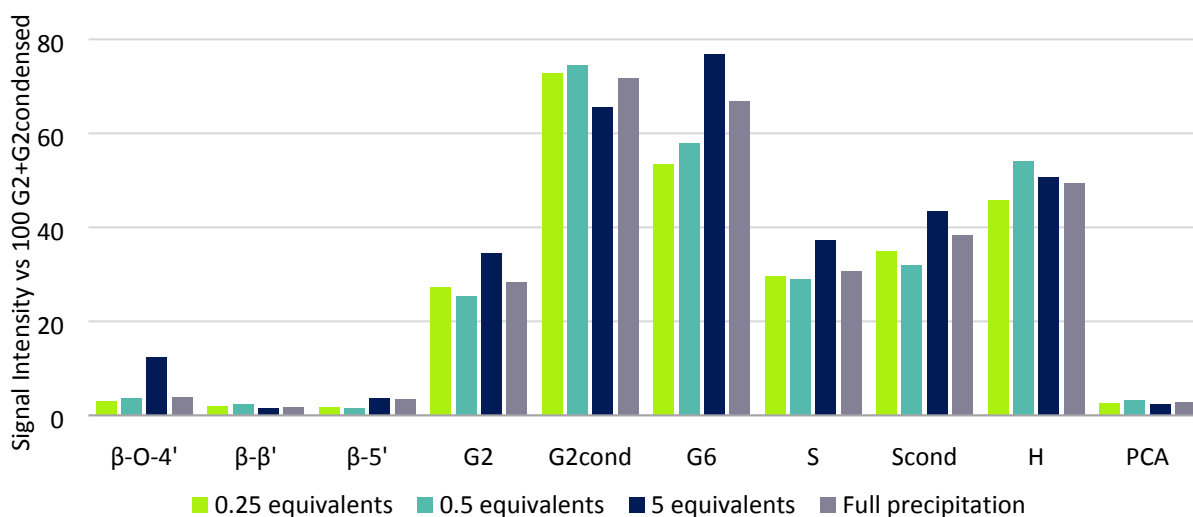
44 For *Miscanthus*, the presence of excess acid accelerated condensation reactions, seen  
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47 in sharper increase in G<sub>6</sub> and G<sub>2</sub> signals and decrease in G<sub>2cond</sub> signals in 5 eq fractions  
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50 compared to initial precipitates (Figure 3b). The effect of acid addition was to exacerbate  
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53 the trends observed, producing lignin fractions with more distinct characteristics, while  
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lignins were on average more condensed. This is consistent with GPC profiles where initial fractions precipitated from excess acid *Miscanthus* treatment was observed to have extremely high molecular weight.

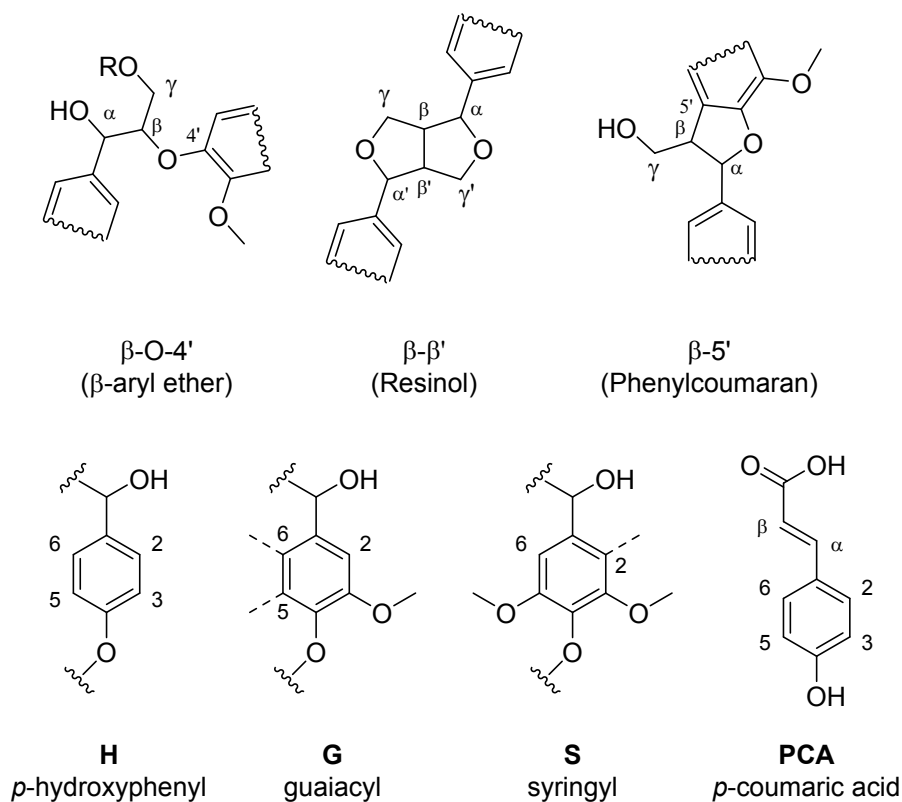
(a) *Miscanthus* treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1



(b) *Miscanthus* treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1



**Figure 3.** HSQC NMR signal intensity of interunit linkages and subunits in lignin isolated from *Miscanthus* as a function of fractional water addition for (a) [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and (b) [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.



**Figure 4.** Structures of the lignin interunit linkages  $\beta$ -O-4',  $\beta$ -5' and  $\beta$ - $\beta'$ ; and the lignin subunits H (*p*-hydroxyphenyl), G (guaiacyl), S (syringyl) and *p*-coumaric acid (PCA).

### Functional Group Analysis by <sup>31</sup>P NMR

The lignin chemical structure was further probed by <sup>31</sup>P NMR analysis, which accurately resolves abundance of hydroxyl moieties, a key functional group that influences lignin

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3 solubility<sup>31</sup> and an important parameter for many lignin valorisation pathways<sup>25</sup>.  
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6 Quantitative integrals from <sup>31</sup>P NMR analysis are displayed in Figure S3 in the  
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10 Supplementary Information. These showed an increase in all hydroxyl group contents  
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13 with fractional precipitation for *Miscanthus* and pine. An exception was seen for willow  
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16 lignin, where initial precipitate (eq = 1) had an unexpectedly high hydroxyl content across  
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19 all groups; this phenomenon appears to be an outlier and is under further investigation.  
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24 The increase of hydroxyl content with fractionation was intensified by addition of excess  
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27 acid. When comparing the final precipitate (5 eq) with initial precipitates, the total hydroxyl  
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30 group content increased around 40% for [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and 60% for  
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33 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1. These trends are consistent with those of previous  
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36 sequential solvent extraction, ultrafiltration and anti-solvent precipitation studies, whereby  
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39 phenolic hydroxyl and total hydroxyl groups showed a good correlation with decreasing  
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42 molecular size<sup>2,4,8,11,13,14,32,33</sup>. Fractions with higher hydroxyl group content are less  
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45 hydrophobic, causing them to stay in solution for longer and be separated from more  
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48 condensed macromolecules (which would also have fewer free end groups due to greater  
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51 extent of recombination by condensation reactions) by fractional precipitation.  
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## Elemental composition

The CHNSO composition of lignins is helpful to assess lignin purity and IL contamination<sup>31</sup>. Significant concentrations of nitrogen and sulfur (presumably derived from the alkylammonium cation and hydrogen sulfate anion, respectively) were detected, amounting to IL contamination of 0.2–13.6 wt% for *Miscanthus* lignins treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1, from 3.9–26.2 wt% for those treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1, from 9.7–25.6 wt% for willow lignins and from 21.1–25.9 wt% for pine lignins. All lignin precipitates were washed four times with deionized water to remove residual IL; however this method has been shown to be imperfect<sup>31</sup>. Contamination tended to be worse for excess acid-treated lignins with very high molecular mass, suggesting that these macromolecules may be trapping IL molecules in the lignin matrix. However, given the size difference between IL molecules ( $M_R = 199$ ) and lignin molecules ( $M_R \sim 1000$ – $200,000$ ) and non-overlap of IL and lignin resonances, IL contamination is *not* believed to affect the trends observed in GPC, HSQC and <sup>31</sup>P NMR analysis.

The CHO content of the “IL-free” portion of the lignin samples was calculated by removing the contribution from N and S components. The corrected elemental carbon content, molar H/C and O/C ratios and estimated higher heating value (calculated from Dulong’s formula<sup>26</sup>) for *Miscanthus* are displayed in Table 3, whereas the same information for willow and pine can be found on the ESI (table S5). While the *Miscanthus*, willow and pine lignin samples pretreated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 indicate a possible trend of decreasing elemental carbon content of the IL-free lignin, which would agree with NMR data indicating initial precipitates were more condensed (i.e. where C–O bonds have been replaced with C–C bonds), no trend could be discerned in the excess acid-treated lignin. The estimated higher heating value (HHV) of lignins did not show any significant variation across the lignin fractions, possibly as the lignins are so

overtreated and condensed that differences in chemical composition between larger and smaller molecular mass lignins cannot be distinguished.

**Table 3.** Elemental carbon, molar H/C and O/C ratios and estimated higher heating value

(HHV) of lignin fractions from *Miscanthus* ([DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1:1 and

[DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1), .

IL	Water	C (wt%)	H/C (mol/mol)	O/C (mol/mol)	HHV (MJ kg <sup>-1</sup> ) <sup>b</sup>
<i>Miscanthus</i> [DMBA][HSO <sub>4</sub> ] <sub>80wt%</sub> a:b=1:1	0.5 eq	63.0±1.0	0.74±0.09	0.39±0.01	21.6
	1 eq	63.3±0.1	0.93±0.02	0.38±0.00	23.3
	5 eq	61.9±0.2	0.90±0.20	0.41±0.01	22.2
	Full precipitation	62.7±0.9	0.94±0.03	0.39±0.02	23.0
<i>Miscanthus</i> [DMBA][HSO <sub>4</sub> ] <sub>80wt%</sub> a:b=1.03:1	0.25 eq	61.5±2.6	0.99±0.10	0.41±0.05	22.7
	0.5 eq	62.7±2.8	0.92±0.10	0.39±0.05	22.8
	5 eq	61.9±0.3	0.98±0.01	0.40±0.01	22.8
	Full precipitation	63.7±0.2	0.91±0.01	0.37±0.00	23.4

<sup>a</sup> All values reported on an IL-free basis after subtracting the contributions from [DMBA][HSO<sub>4</sub>] contamination as determined via N and S contents.

<sup>b</sup> Estimated using Dulong's formula<sup>26</sup>.

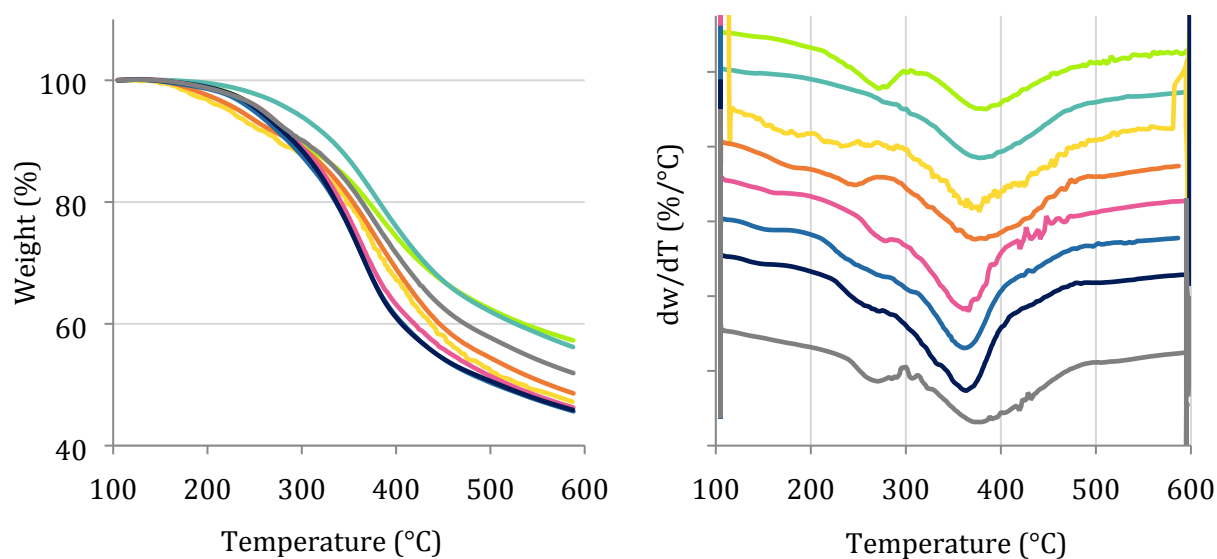
### Thermal properties by TGA and DSC

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3 Thermal behavior of lignins is key for its utilization as a fuel, carbon fiber precursor, and  
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6 thermochemical valorization by pyrolysis or gasification<sup>34–36</sup>. For instance, carbon fiber  
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9 production requires low ash content and high char residue to improve melt-spinning<sup>37</sup>.

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13 The effect of fractionation on lignin thermal properties was evaluated according the  
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16 thermal degradation of *Miscanthus* lignins treated with [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1,  
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19 due to the wide variation in molecular size across lignin fractions.  
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24 The thermograms and differential thermograms from TGA (Figure 5) showed the first  
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27 mass loss at ~200 °C for all lignin fractions. Later precipitates (1.5, 2, 2.5 and 5 eq) began  
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30 to degrade at a slightly lower temperature, as they are mainly composed of less  
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33 condensed components with lower molecular mass and more thermally labile. The next  
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36 thermal degradation event occurred around 300–400 °C, where the initial precipitates  
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39 (0.25, 0.5 and 1 eq) displayed a more gradual degradation pattern, in line with their higher  
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42 polydispersity. Lignin degradation occurred in a broad temperature range, starting from  
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45 300 °C when aliphatic side chains start to break off to cleavage of more thermally stable  
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48 C–C bonds at 370–400 °C<sup>38</sup>. The residual mass at 600 °C, attributed to non-volatile char  
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51 and ash residues, increased for higher molecular mass lignin fractions (as 5 → 0.25 eq).  
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3 Decreasing thermal stability and decreasing residual char for later precipitates was also  
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6 reflected in decreasing volatile matter to fixed carbon (VM/FC) ratios (Table 4 and Table  
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10 S11), agreeing with results of previous works on Kraft fractionation<sup>9–11,39</sup>. The VM/FC ratio  
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12 indicates the proportion of lignin material converted to volatile matter compared to char  
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17 residue during thermal degradation. Based on the DTG curve, the temperature of maximal  
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20 thermal degradation also shifted from 363 to 379 °C between 5 and 0.25 eq, the latter  
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23 matching the DTG<sub>max</sub> value of full precipitate (382 °C). This trend of increasing thermal  
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27 stability with molecular size, while expected from the literature<sup>4,13,14,39,40</sup>, also corresponds  
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31 with the lower abundance of thermally labile aryl ether linkages and phenolic hydroxyl  
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34 groups seen in HSQC and <sup>31</sup>P NMR<sup>9</sup>.  
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**Figure 5.** Thermograms (TG) and differential thermograms (DTG) of fractions obtained by fractional precipitation of *Miscanthus* lignins using [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.

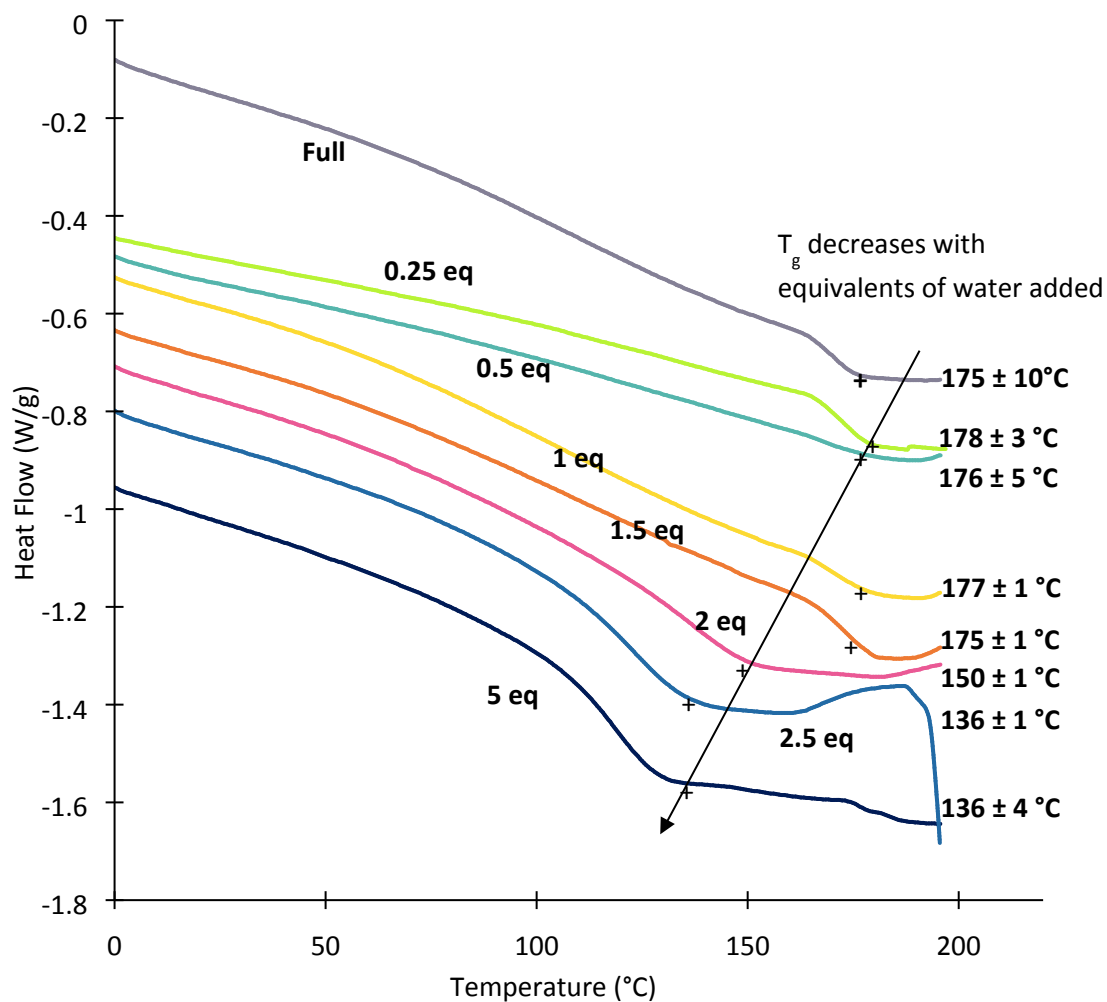
**Table 4.** Peak lignin degradation temperature (DTG<sub>max</sub>) and volatile matter to fixed carbon ratio (VM/FC) on a dry ash-free basis for lignin fractions isolated from *Miscanthus* using [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.

Eq of water	DTG <sub>max</sub> (°C)	VM/FC (wt/wt)
0.25	379.3±1.4	0.87±0.07
0.5	377.9±0.7	1.03±0.16
1	370.0±0.7	1.15±0.18
1.5	364.7±1.8	1.24±0.00
2	362.9±0.7	1.28±0.10
2.5	360.6±0.8	1.38±0.00
5	362.5±0.4	1.40±0.01
Full precipitation	382.2±0.4	1.08±0.04

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3 The glass transition temperature ( $T_g$ ), which can be observed by DSC, is an essential  
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6 parameter for lignin processing in various applications; for example, when lignin is used  
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9 as a precursor for carbon fiber production, the  $T_g$  strongly impacts the melt-spinning<sup>34</sup>.

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13 Figure 6 shows the DSC thermograms for *Miscanthus* lignins isolated using  
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16 [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1, wherein individual lignin fractions were most distinct so  
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19 differences in thermal properties could be easily observed. The samples were subjected  
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22 to an initial scan (above their  $T_g$ ) to eliminate the stored thermal history of the polymers.  
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27 A distinct glass transition event could be observed for all samples, though this was most  
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30 sharp for the later precipitates (2, 2.5 and 5 eq) which are less polydisperse.  $T_g$  decreased  
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33 from 178 °C for 0.25 eq to 136 °C for 5 eq, indicating a decrease in thermal stability with  
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36 increasing addition of water equivalents.  $T_g$  can be influenced by several molecular factors  
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39 such as molecular weight, crosslinking, branching and intermolecular forces. The  
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42 decrease in  $T_g$  with further sequential fractionation can be indicative of a less condensed  
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45 structure with fewer C–C bonds (seen by HSQC NMR). However, the higher phenolic OH  
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48 content witnessed by <sup>31</sup>P NMR seems to contradict this, as these groups form stronger  
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51 hydrogen bonds which would have the effect of increasing  $T_g$ .  
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**Figure 6.** DSC thermogram showing the glass transition of fractionated and full precipitate of *Miscanthus* lignins isolated using [DMBA][HSO<sub>4</sub>]<sub>80wt%</sub> a:b=1.03:1.

The ability to fractionate lignins into distinct fractions with specific molecular size and thermal stability is a major benefit of the proposed anti-solvent fractionation scheme. The higher thermal stability found in lignins precipitated with lower equivalents of water is likely attributable mostly to their very high molecular weight.

## CONCLUSIONS

IonoSolv lignins were fractionated by sequential addition of water as anti-solvent to IL-water mixtures. The resulting fractions were more homogeneous than lignin obtained in a single precipitation step. Addition of 1 g of water per g of dry IL in the liquor was able to precipitate up to 90 wt% of lignin fragments compared to the lignin mass isolated by excess water addition (5 g/g). Fractionation appeared to be controlled by molecular weight of lignin polymers. *Miscanthus* lignins obtained using [DMBA][HSO<sub>4</sub>] mixtures with stoichiometric acid:base ratios and excess acid were highly monodisperse with polydispersity index of 2–3, and low molecular weight fractions around ~1000 g mol<sup>-1</sup> could be isolated by fractional precipitation in yields of 5–10 wt%. Precipitates isolated with increasing amounts of anti-solvent (i.e. decreasing dissolution power of the solvent mixture) possessed (1) low molecular weight and narrow molecular weight distribution, (2) high phenolic and total hydroxyl content, (3) low degree of condensation, (4) low carbon content and (5) reduced thermal stability, compared to the full precipitate. The presence of excess acid in the IL solvent exacerbated the differences between lignin fractions and produced more condensed and high molecular weight lignin fractions.



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3 Sequential precipitation of ionoSolv lignins could be used to tune the lignin quality and process  
4 cost. It allows lignin fractionation and recovery in a single process step (and one necessary for  
5 solvent recycling anyway) *i.e.* high molecular weight fractions with high thermal stability may  
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7 be suitable for production of activated carbons or carbon fibers<sup>40</sup>; whereas precipitates with low  
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9 molecular weight, narrow  $M_w$  distribution and high hydroxyl content could be used in polymeric  
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11 or adhesive applications<sup>3,41</sup>.  
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## 17 ASSOCIATED CONTENT

18  
19  
20 Supporting Information. All chemical synthesis and pretreatment procedures; analytical  
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22 procedures and analysis results for biomass compositional analysis and lignin  
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24 characterization by GPC, 2D HSQC NMR, <sup>31</sup>P NMR, elemental analysis, and proximate  
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26 analysis.  
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### Author Contributions

48  
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50 The manuscript was written through contributions of all authors. All authors have given approval  
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52 to the final version of the manuscript.  
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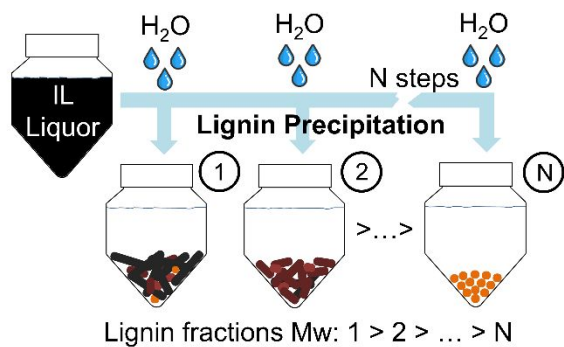
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## 10 ABBREVIATIONS

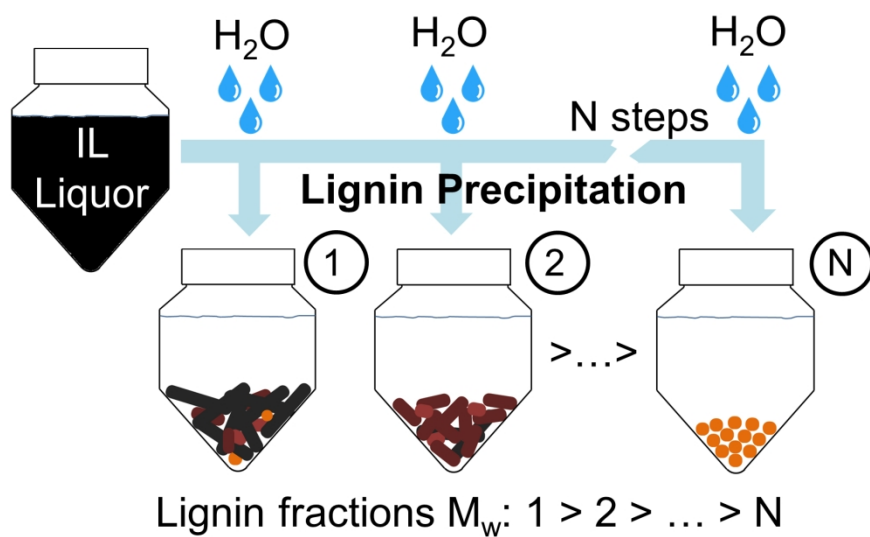
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13 [DMBA], *N,N*-dimethylbutylammonium; [Hbim], *N*-butylimidazolium; [TEA],  
14 triethylammonium; aq., aqueous; DSC, differential scanning calorimetry; DTG,  
15 differential thermogram; DTG<sub>max</sub>, maximum point of differential thermogram; EA,  
16 elemental analysis; eq, equivalent; FC, fixed carbon; IL, ionic liquid; GPC, gel  
17 permeation chromatography; HHV, higher heating value; HPLC, high performance liquid  
18 chromatography; HSQC, heteronuclear single quantum coherence; M, molar  
19 concentration; M<sub>n</sub>, number average molecular weight; M<sub>w</sub>, weight average molecular  
20 weight; NMR, nuclear magnetic resonance spectroscopy; PDI, polydispersity index; T<sub>g</sub>,  
21 glass transition temperature; TGA, thermogravimetric analysis; VM, volatile matter; wt%,  
22 weight percent.  
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## 49 GRAPHICAL ABSTRACT



## NONTECHNICAL SYNOPSIS

Lignin was separated into fractions with different properties by sequential addition of small amounts of water to ionic liquid post-pretreatment.



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