



Comparing multistage liquid–liquid extraction with cold water precipitation for improvement of lignin recovery from deep eutectic solvents

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ABSTRACT

After biomass fractionation using deep eutectic solvents (DES), solvent recovery is an essential step. Recovery of lignin from DES by liquid–liquid extraction (LLX) may provide large energy savings compared to cold water precipitation. Lignin that is dissolved in DES from biomass fractionation is inhomogeneous, meaning it has various fractions with different molar weights and possibly variations in functional group densities. Therefore, it is important to compare recoverability of every lignin fraction by LLX with cold-water precipitation. In this work, the recovery of lignin from a DES comprised of 30 wt% choline chloride and 70 wt% lactic acid was studied. Using as much as 30 wt% choline chloride is beneficial to limit leaching of lactic acid. Three cross-current extractions were performed using 2-MTHF. This method recovered 95% of the lignin fractions around 2000 g/mol and 85% of the lignin fractions around 10,000 g/mol. No inter-aromatic ether bonds were found in the lignin remaining in the DES raffinate by heteronuclear single quantum coherence spectroscopy (HSQC), indicating the remaining lignin in the DES has a highly condensed nature. Cold water precipitation could fully recover the lignin fractions above 4000 g/mol using 3.5 kg water per kg DES. However, only half of the lignin fraction of 1000 g/mol was recovered. Briefly, LLX is more suitable for the recovery of low molar weight fractions, while cold water precipitation is more suitable for the heavy molar weight fractions. For industrial applications, a combination of both approaches is essential for full lignin recovery.

1. Introduction

Deep eutectic solvents (DESs) are composite solvents that exhibit deep eutectic behavior upon mixing the constituents. One attempt to define DESs states that the melting points of these mixtures is reduced considerably more ($> 50\text{ }^{\circ}\text{C}$) than would be the case for ideal mixtures [12]. Many mixtures that have been reported as being DESs do have a much lower melting point than the pure components. However, these melting points are often close to ideal melting points that can be calculated thermodynamically [3]. Furthermore, the melting points determined for these mixtures may differ significantly upon the methods used. For example, the melting point of the DES comprised of choline chloride and lactic acid in a 1:10 ratio was determined by Crespo et al. [3], and by Francisco et al. [4] Crespo et al. [3] found the melting point to be close to the ideal melting point of $11\text{ }^{\circ}\text{C}$ using a capillary method, while Francisco et al. [4] found a melting point of $-66\text{ }^{\circ}\text{C}$ for the same mixture by differential scanning calorimetry (DSC). Possibly, the results obtained by the DSC method used by Francisco et al. [4] deviate

because of supercooling in the DSC [5]. Furthermore, the hygroscopic nature of DESs may also make characterization difficult since water strongly influences phase behaviour [6].

Regardless of what a formal definition of DESs should be, these composite solvents that exhibit their low transition temperatures [7] due to hydrogen bonding, raised wide attention in academia. Since these solvents can easily be prepared in numerous ways by combining a hydrogen bond donor and acceptor [8] and are often biocompatible, biodegradable [9] and can have a low toxicity [10], DESs have been used for various applications, such as, CO_2 capture [11–13], air pollutant removal [14], extractive distillation [15], metal extractions [16], desulfurization [17,18] and biomass fractionation [19–22].

Regeneration of DESs is most often performed by precipitation of either the solute [23,24] or solvent [25,7] in an anti-solvent. Especially in biomass fractionation this approach is popular, but large amounts of water are required as anti-solvent for the precipitation of lignin [26]. In our previous study [27], we proposed LLX using hydrophobic solvents to recover lignin from DES, since LLX can be energy efficient [28]. It

Abbreviations: BHT, butylated hydroxytoluene; DES, deep eutectic solvent; FT-IR, Fourier-transform infrared spectroscopy; GPC, gel permeation chromatography; HSQC, heteronuclear single quantum coherence spectroscopy; LLX, Liquid-liquid extraction

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was found that 2-MTHF is a suitable extraction solvent to recover lignin from a DES comprising of lactic acid and choline chloride from single stage equilibrium extraction studies [27]. This solvent was selected because it could extract lignin of all molar weights and because it is bio-based [27]. In precipitation, an anti-solvent is added to the DES, which decreases the affinity of lignin with the mixture, causing the lignin to precipitate. This antisolvent must be removed from the DES before reuse of the DES. In LLX, the DES is contacted with an immiscible solvent. If the lignin has a higher affinity to the solvent than to the DES, it will move from the DES to the solvent phase. The solvent can later be removed from the lignin by a simple evaporation step.

Lignin is an inhomogeneous polymer and contains various linkages and functional groups. Various authors used either LLX [29–31], fractional precipitation [32–34], or partial solubility [32,35–39] to fractionate a lignin in two or more fractions with different properties. This means that some lignin fractions may be better extracted and/or precipitated from the DES than others. Therefore, it is vital to determine to what extent different lignin fractions can be recovered from DESs. Compared to the prior study, where only single stage extractions of lignin were described, we here describe a more extensive study on multistage cross-current LLX to recover lignins from a lactic acid and choline chloride mixture. We applied the same DES composition (30 wt % choline chloride and 70 wt% lactic acid) as in the previous study [27], this composition is beneficial to limit lactic acid leaching. At lower choline chloride compositions, leaching of lactic acid can become much more significant. For future industrial perspective, lactic acid leaching, even when limited by the use of 30 wt% choline chloride, should be considered. After evaporative regeneration of the 2-MTHF, the lactic acid is recovered from lignin by a water wash. Furthermore, we compared the multistage extraction method to cold water precipitations using various amounts of cold water. The results in this paper give more in-depth insight in the extractability of the lignin fractions, and how full lignin removal can be approached.

In this study, lignin extractions from a DES comprised of lactic acid and choline chloride using 2-MTHF were studied in a cross-current sequence, and results for lignin-in-DES solutions obtained via different procedures were compared. In our studies we always used *Eucalyptus globulus* as source for the lignin, but the preparation of the lignin containing DES was varied. Lignin was extracted directly from the prepared lignin-in-DES mixture, and lignin that was previously obtained by precipitation was redissolved in the DES and then recovered again. The lignin concentrations in the extracts were determined by gel permeation chromatography (GPC). Later, the lignin fractions were isolated from all extract and raffinate streams and characterized by Fourier-transform infrared spectroscopy (FT-IR) and HSQC to compare the general structure and bonds in the lignin fractions. Also, the recoverability of different lignin fractions by cold water precipitation was studied as function of the water to DES phase ratio using GPC.

2. Methods and materials

2.1. Chemicals

Lactic acid (> 90%) and 2-MTHF (Emplura) were ordered from VWR. Choline chloride (> 98%) and butylated hydroxytoluene (BHT) (> 99%) were ordered from Sigma-Aldrich. Crystalline lactic acid was kindly provided by Corbion. DMSO- d_6 (D, 99.9%) was ordered from Cambridge Isotope Laboratories. Air-dry *Eucalyptus globulus* chips were kindly provided by The Navigator Company. The commercial size chips (typically 25–35 × 10–25 × 2.5–6 mm, L × W × T) were used as received and contained 21.6% lignin, 50.6% glucose, 14.0% xylose, and 1.1% galactose, as determined by acid hydrolysis using the standard NREL method [40].

2.2. Experimental methods

2.2.1. Preparation of lignin-in-DES mixtures

210 g Choline chloride, 490 g lactic acid (> 90%) and 300 g water were added to a round bottom flask fitted with a condenser. The mixture was heated under continuous stirring to form a homogeneous liquid, which started to boil at 112 °C. Water was released from the mixture until the temperature reached 120 °C and 50 g wood chips (oven dry basis, 10.9 wt% water) were added through a free neck (ø 29 mm). The mixture was kept at 120 °C for 8 h under continuous stirring. The mixture was filtered while hot over a 53 µm steel mesh and contained 17.4 wt% water, as determined by Karl-Fischer titration (see Section 2.3.4).

2.2.2. Cross-current extraction experiments

20 g of the prepared lignin-in-DES mixture (see Section 2.2.1) and 20 g 2-MTHF were added to a 50 mL double-walled stirred cell, which was kept at 50 °C using a Julabo F25 water bath. The cell was stirred over night to reach equilibrium. After extraction, the phases were settled and the top phase was removed and renewed by a solution of 30 wt % crystalline lactic acid in 2-MTHF to avoid excess leaching of lactic acid. The lignin concentrations in the extract phases were determined by GPC. The lignin concentrations in the raffinate phases were calculated by mass balance since they were not fully soluble in the GPC eluent. The distribution coefficients were calculated as the ratios between the concentrations in the extract phases, over the concentrations in the raffinate phases, and the recoveries were calculated by mass balance.

Also three stage extractions with lignin that was obtained by cold water precipitation according to the method described in our previous paper [27] were performed. 15 g Crystalline lactic acid, 8 g choline chloride and 15 g 2-MTHF were added to the stirred cell described in the previous section. The mixture forms 18 g of extract and 18 g of DES raffinate (comprised of 30:70 w:w choline chloride to lactic acid), as determined from the equilibrium [27]. 6 g water was added to the DES to adjust the initial water content in the DES to 25 wt%, being beneficial for the distribution of lignin [27]. 300 mg lignin was added to the stirred cell and the mixture was stirred over-night to reach equilibrium. Three extractions were performed using the same method as described in the previous paragraph. The lignin concentrations in the extract and raffinate phases were determined by GPC. The distribution coefficients were calculated as the ratios between the concentrations in the extract phases, over the concentrations in the raffinate phases, and the recoveries were calculated by mass balance according to:

$$\text{Recovery (\%)} = \frac{\sum_{i=1}^n C_{\text{raffinate},i} \cdot m_{\text{raffinate},i}}{C_{\text{DES,initial}} \cdot m_{\text{DES,initial}}} \cdot 100$$

In which C is the lignin concentration, m the mass of the phase and n the extraction stage.

2.2.3. Isolation of lignin fractions

To obtain sufficient lignin from each fraction for characterization, an additional set of extractions were performed on larger scale using a modified procedure. For the isolation of the lignin from the first raffinate and extract phase, 100 g lignin-in-DES mixture and 100 g 2-MTHF were added to a separatory funnel. The mixture was shaken vigorously for several minutes and the phases were settled and separated at room temperature. A minor amount of precipitate was found on the interface between the extract and raffinate phase. For further characterization of this fraction, please see the Electronic Supplementary Information (ESI). 2-MTHF was removed from the extract phase by rotavap at 50 °C and 200 mbar until no further 2-MTHF evaporated from the mixture. Both phases were added to 300 mL water under stirring to precipitate the lignin. The lignin fractions from the extract and raffinate phases and

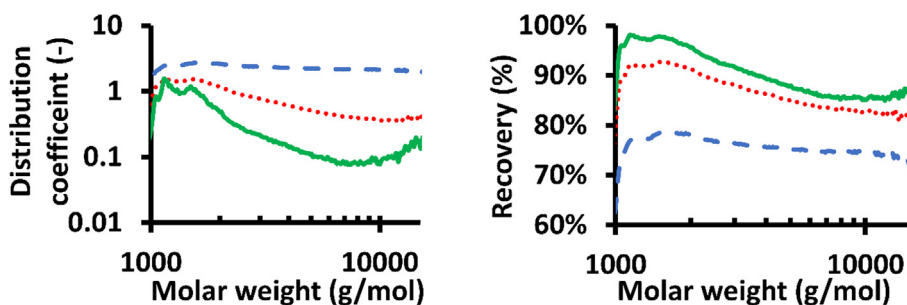


Fig. 1. Results of the cross-current extractions of lignin from the lignin-in-DES solution by 2-MTHF in 3 stages (Stage 1 is dashed, 2 dotted and 3 solid lines). A 1:1 DES to solvent ratio was used and extractions were performed at 50 °C. The initial DES contained 17.6% water. The left figure shows the distribution coefficients and the right figure shows the total lignin recovery, as calculated by mass balance.

the precipitate found between the two fractions were separated by centrifuge, washed twice with 20 mL water and dried overnight under vacuum.

For the isolation of the second and third raffinate and extract phases, 330 g lignin-in-DES mixture and 330 g 2-MTHF were added to a separatory funnel. The mixture was shaken vigorously for several minutes and the phases were settled and separated at room temperature. After the extraction, 190 g DES raffinate and 450 g 2-MTHF extract were recovered and a minor amount was lost during separation. All raffinate was added to 190 g of 30 wt% crystalline lactic acid in 2-MTHF. The mixture was shaken vigorously for several minutes and the phases were settled and separated. 190 g DES raffinate and 165 g 2-MTHF extract were obtained. 100 g of this raffinate was added to another 100 g of the 30 wt% crystalline lactic acid in 2-MTHF mixture. The mixture was shaken vigorously for several minutes and the phases were settled and separated. 100 g DES raffinate and 90 g 2-MTHF extract was obtained. 2-MTHF was removed from both raffinates by rotavap at 50 °C and 200 mbar until no further 2-MTHF evaporated from the mixture. All raffinates and extracts were precipitated in excess (1:3 w:w) water. The lignin fractions from the extracts and raffinates were separated by centrifuge, washed twice with 20 mL water and dried overnight under vacuum. Insufficient lignin was recovered from the 3rd 2-MTHF extract for analysis.

2.2.4. Cold water precipitation

The lignin-in-DES mixture (see Section 2.2.1) and water were both mixed at room temperature in various ratios to form a total of 1 g in a 1.5 mL Eppendorf centrifuge tube. The mixtures were shaken thoroughly and left over night at room temperature. The solids were separated by centrifugation at 14,000 rpm for 10 min. The lignin content in the supernatant was determined by GPC.

2.3. Analytical methods

2.3.1. GPC

An Agilent 1200 series was used with a refractive index detector and a UV detector operating at 254 nm using 3 GPC PLgel 3 μ m MIXED-E columns in series. The column was operated at 40 °C and a 95:5 (v:v) tetrahydrofuran and water mixture was the solvent at a flowrate of 1 mL/min. Molecular weight distributions were calibrated using polystyrene solutions having molecular weights ranging from 162 to 27,810 g/mol.

2.3.2. HSQC

Lignin samples were dissolved in DMSO- d_6 and analyzed using a Bruker Ascend 400 MHz spectrometer. Data was acquired using the hsqcetgp pulse program provided by Bruker. Matrices of 512 data points for the ^1H -dimension and 256 data points for the ^{13}C -dimension were collected applying a relaxation delay of 1.5 s and spectral widths from -1 ppm to 11 ppm and from 210 to 0 ppm in the ^1H and ^{13}C dimensions, respectively. The 2D HSQC spectra were processed using MestReNova software.

2.3.3. FT-IR

FT-IR analyses were performed on solid lignin fractions isolated from the raffinates and extracts. The analysis was carried out at room temperature using a Bruker Tensor 27 spectrometer equipped with an attenuated total reflection system and a deuterated triglycine sulphate detector. Absorbance was measured in the range 650 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} , and 16 scans were performed. No baseline corrections were applied.

2.3.4. Karl-Fischer titration

The water content of lignin in DES mixture was determined by Karl-Fischer titration using a Metrohm 787 KF Titrino. Hydranal composite 5 (5 mg water/mL) was titrated from a 20 mL burette in a 3:1 (v:v) mixture of methanol and dichloromethane. The sample was measured in duplo with a relative error < 1%.

3. Results and discussion

3.1. Cross-current extractions using actual pulping DES

Cross-current extractions were performed to investigate the extractability of lignin from the prepared lignin-in-DES mixture. Three stage extractions were performed using 2-MTHF. The lignin concentrations in the solvent phases were determined by GPC and the concentrations in the DES phases were calculated by mass balance. From these concentrations the distribution coefficients of lignin among the 2-MTHF and DES were calculated, as well as the total recovery of lignin from the DES (see Section 2.2.2). The results are shown in Fig. 1. From this figure, it can be seen that the total lignin recovery after 3 extraction stages was approximately 95% for the fractions around 2,000 g/mol, and decreased to around 85% for the fraction around 10,000 g/mol. The distribution coefficient in the first stage was around 2.5 for all lignin fractions, dropped below 1 in the last stage. Therefore, further extraction stages seemed ineffective. Because the lignin remaining in the DES could not be extracted by 2-MTHF, we conjecture that the remaining lignin has a different chemical nature compared to the lignin that was extracted by 2-MTHF. Therefore, lignin from each fraction was isolated using a modified procedure for FT-IR and HSQC analysis to elaborate on their structures (see Section 3.3).

3.2. Cross-current extraction using precipitated lignin

In the previous section, lignin was extracted directly from the prepared lignin in DES mixture. However, this mixture does not only contain lignin, but also (hemi)cellulose breakdown products, such as glucose, xylose, furfural, HMF or humins. These substances may influence the lignin distribution, and therefore, the extraction experiments from Section 3.1 were repeated using lignin that was previously obtained by cold water precipitation, to see whether extraction of a model lignin produced by the same pulping procedure is representable for the actual system. However, some lignin fractions may remain soluble during cold water precipitation (see Section 3.4), and are therefore not present in the precipitated lignin used in the previous section. This

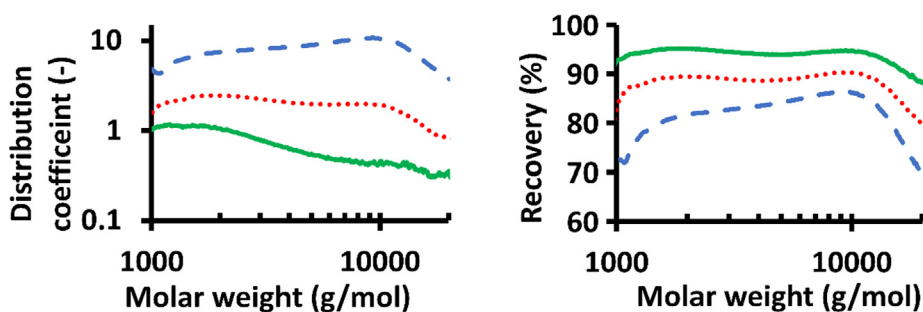


Fig. 2. Results from the cross-current extractions of lignin by 2-MTHF from a DES comprised of lactic acid and choline chloride in 3 stages (Stage 1 is dashed, 2 dotted and 3 solid lines). Extractions were performed at 50 °C using a 1:1 DES to solvent ratio. The initial DES contained 25% water. The left figure shows the distribution coefficients and the right figure shows the total lignin recovery, as calculated by mass balance.

means that the distribution of these fractions cannot be measured in this experiment. The results from this experiment are shown in Fig. 2. From this figure it can be seen that the distribution coefficient in the 1st stage is between 4 and 10, which is in accordance with the earlier determined equilibrium [27]. In the 3rd stage, the distribution coefficient dropped to a value lower than 1 for all lignin fractions. This was also seen in the direct extractions from the lignin-in-DES mixture. The lignin recovery after 3 stages of the fractions between 1000 and 10,000 g/mol was around 95%, while the recovered dropped to 85% for the lignin fractions around 20,000 g/mol.

The main difference between the total recovery of lignin extracted directly from the lignin-in-DES mixture and the lignin that was previously obtained by precipitation can be found in the region between 3000 and 10,000 g/mol. For the first lignin, the recovery drops from 95% to 85% between these molar weights, while for the second lignin (previously precipitated) the recovery remains constant around 95% and only drops to 85% at a molar weight around 20,000 g/mol. However, for both lignins the full recovery for the lowest molar weight fraction is around 95% and the recovery of the highest molar weight fraction around 85%, indicating that the lignin model is reasonably representative for the actual lignin-in-DES mixture.

3.3. Analysis of DES fractions

In the previous sections it was shown that the lignin extractability from DES decreased one order of magnitude in the 3rd extraction stage, compared to the 1st stage. We conjectured that this was caused by a difference in chemical nature between the lignin extracted by 2-MTHF and the lignin remaining in the DES. Therefore, we performed larger scale extractions using a modified procedure to be able to isolate enough lignin from each raffinate and extract fraction, in order to study the chemical natures by FT-IR and HSQC. In this procedure, lignin was extracted from the lignin-in-DES mixture using multiple extractions with 2-MTHF using a separatory funnel at room temperature. The lignins were isolated from each raffinate and extract fraction using cold water precipitation.

In theory, FT-IR provides information on all chemical bonds present in a lignin structure [35] and HSQC gives information on the general structure and ether bonds present in lignin [35,41]. These factors may influence lignin solubility and by performing these analysis on both lignin fractions, it may become clear why some lignin fractions are better extracted than others. The FT-IR spectra (see Fig. 3) of the lignins in the DES raffinates and 2-MTHF extracts show only minor differences. There are only minor differences between the spectra of the lignin from the 1st and 2nd DES raffinates, while the spectrum of the lignin in the 3rd raffinate is similar to the 2nd raffinate. Also the spectrum of the lignin in the 1st 2-MTHF extract is similar to the three spectra of the lignins in the three DES raffinates. Some extra peaks appear in the 2nd 2-MTHF extract at 859, 888, 1157, 1133, 1310 and 1358 cm⁻¹. However, all of these peaks can be attributed to the presence of a contamination of BHT in the lignin. The FT-IR spectrum of BHT is shown in the supplementary data. BHT is an additive that is present as stabilizer in 2-MTHF, which was left in the lignin sample after the removal of 2-MTHF. Since the lignin concentration in the 1st extract is

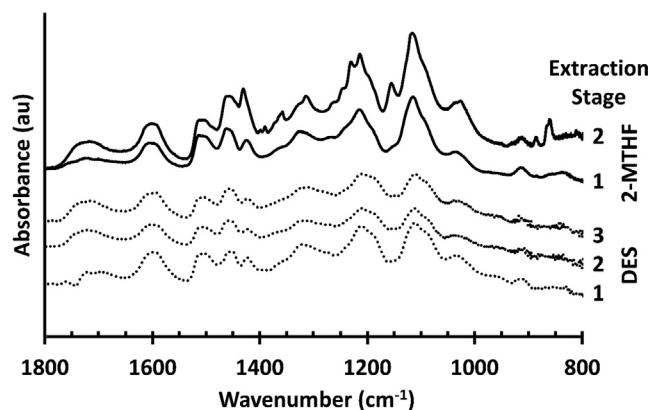


Fig. 3. FT-IR spectra of the lignins isolated from the DES raffinates (dotted) and 2-MTHF extracts (solid).

much higher than in the 2nd extract, and the BHT concentration in 2-MTHF is constant, the BHT contamination in lignin from the 2nd extract is much higher than from the 1st extract. Not enough lignin could be isolated from the 3rd extract phase for analysis.

The HSQC spectra of the lignins in the DES raffinates and 2-MTHF extracts are shown in FigS. 4 and 5. The spectra of the oxygenated side chain region show the presence of ether bonds between the aromatic units. The β -O-4 bonds ($\delta_C/\delta_H = 72.1/4.9$) and β - β bonds ($\delta_C/\delta_H = 85.8/4.7$) can be clearly identified in the spectra of the two 2-MTHF extracts and the first DES raffinate. However, they do not show in the 2nd and 3rd DES raffinate. The β -5 bonds could not be identified in any lignin fraction. Furthermore, the spectra show the methoxy groups in the lignin ($\delta_C/\delta_H = 56.3/3.7$) and impurities of lactic acid ($\delta_C/\delta_H = 69.0/5.0$ and $66.6/4.2$) and choline chloride ($\delta_C/\delta_H = 53.6/3.2$). The spectra of the aromatic region show the presence of guaiacyl ($\delta_C/\delta_H = 115.7/6.8$) and synapyl ($\delta_C/\delta_H = 103.9/6.7$) units in all lignin fractions. Furthermore, the spectra of the two 2-MTHF extracts clearly show the contamination of BHT ($\delta_C/\delta_H = 125.5/6.9$). Reference spectra of all impurities are shown in the ESI.

There are two main differences between the lignin that was extracted by 2-MTHF, compared to the lignin that remained in the DES raffinate. First of all, the lignin that remained in the raffinate has a higher molar weight than the lignin that was extracted by 2-MTHF. Second, the lignins that were extracted by 2-MTHF contained interaromatic ether bonds, such as β -O-4 and β - β bonds, whereas these bonds were not found in the lignin that remained in the DES raffinate. These observations suggest that the lignin that remains in the DES raffinate has a highly condensed nature, compared to the lignin that was extracted by 2-MTHF.

3.4. Recovery by cold water precipitation

The current laboratory benchmark to recover lignin from DES is precipitation of lignin by addition of the DES to water or aqueous mixtures [26]. We also studied the recovery of lignin by cold water

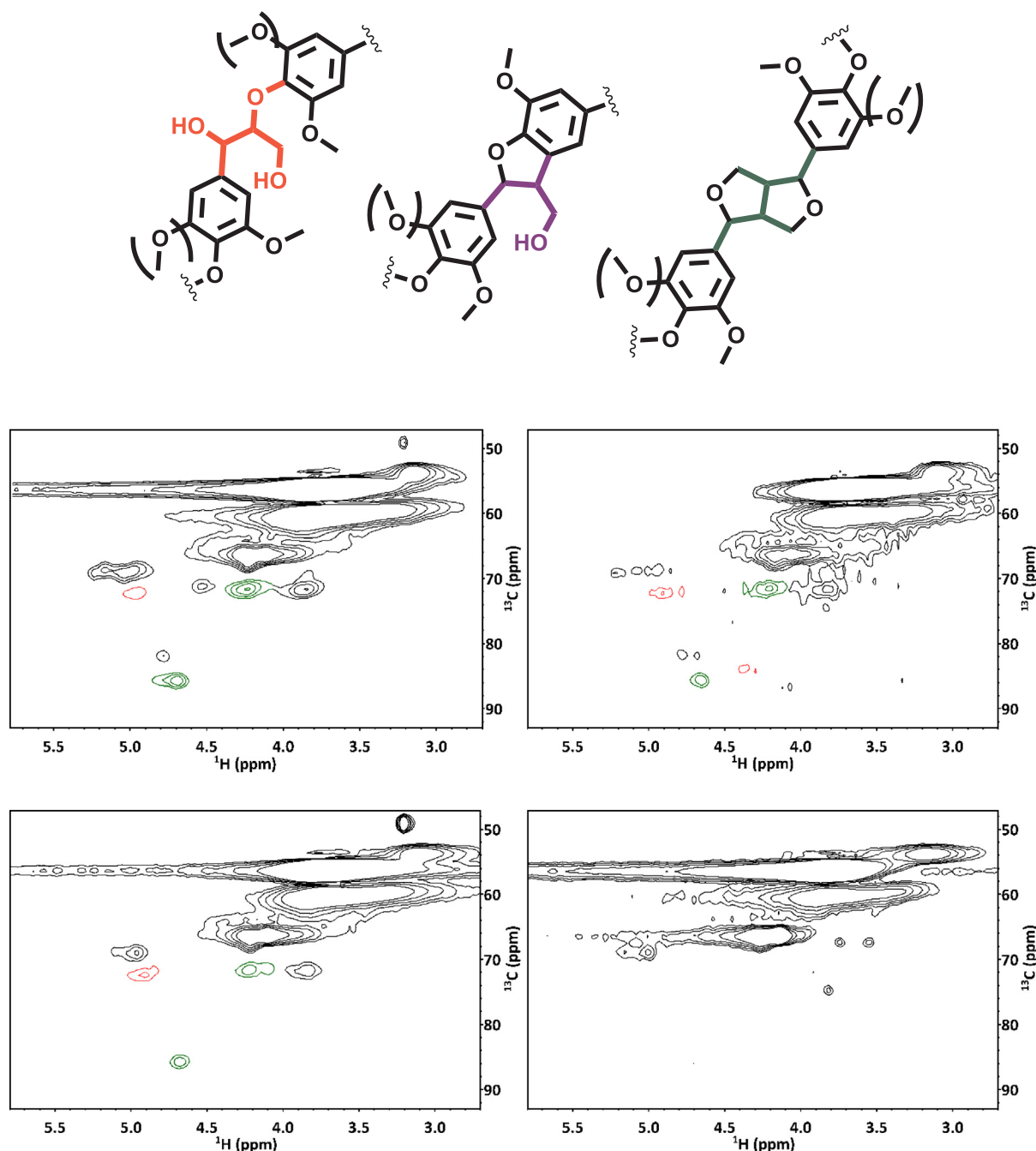


Fig. 4. ^1H - ^{13}C HSQC spectra of the lignin isolated from the DES raffinates and 2-MTHF extracts in the aliphatic oxygenated side chain region ($\delta_{\text{C}}/\delta_{\text{H}} = 47\text{--}93/2.7\text{--}5.8$ ppm). On top of the figures, the structures of the three main inter-aromatic ether bonds are shown: $\beta\text{-O-4}$ (left, red), $\beta\text{-5}$ (middle, purple) and $\beta\text{-}\beta$ (right, green). The peaks identified to these bonds in the spectra are labeled by color ($\beta\text{-5}$ bonds were not found in the spectra). Top left: lignin from the 1st 2-MTHF extract, Top right: lignin from the 2nd 2-MTHF extract, Bottom left: lignin from the 1st DES raffinate, Bottom right: lignin from the 2nd DES raffinate.

precipitation to make a direct comparison between both techniques. Water and the lignin-in-DES mixture were mixed in various ratios. The precipitated lignin was separated by centrifuge and the concentration of lignin remaining in the supernatant was determined by GPC. The recovery was calculated from the lignin concentration in the supernatant and the lignin concentration in the original DES. The results of these experiments are shown in Fig. 6.

From Fig. 6 it becomes apparent that only minor amounts of water are required to precipitate a fraction of the lignin, 0.22 g water per g DES was enough to precipitate roughly half of the lignin fraction around 20,000 g/mol. However, the lower molar weight fraction remained partly soluble in the water-DES mixture. Even when 3.5 g water

per g DES, about half the lignin fraction around 1000 g/mol was recovered from the DES. We reckon that higher water to DES ratios are not viable for industrial application since the added water must be removed from the DES by evaporation, which is highly energy intensive. Therefore, these ratios were not studied in this work.

3.5. Comparison between LLX and cold water precipitation

The recovery of lignin from a DES comprised of lactic acid and choline chloride was studied for two methods, LLX using 2-MTHF and precipitation using cold water. Large differences were found between the two methods. For LLX around 95% of the lignin fraction around

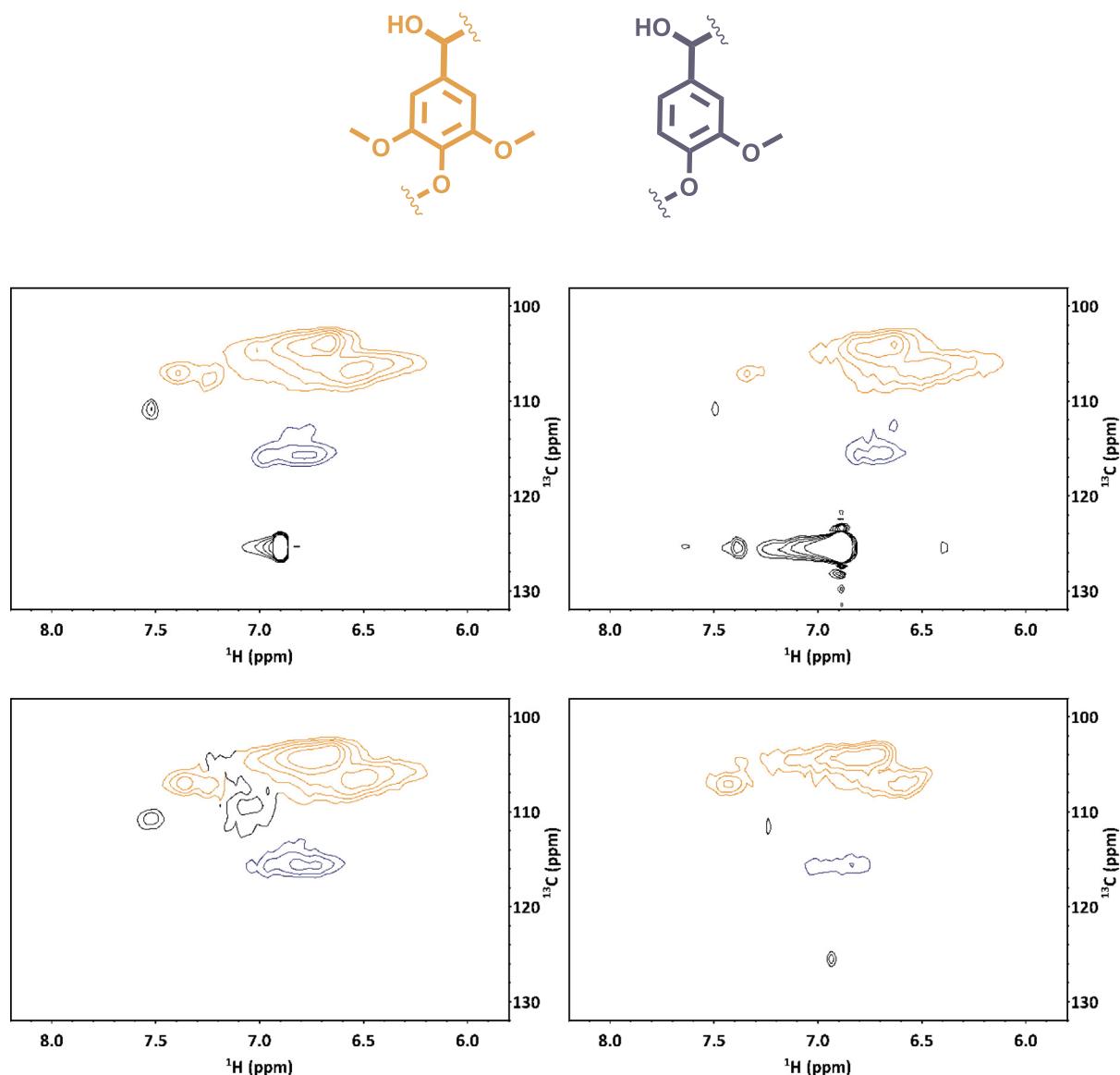


Fig. 5. ^1H - ^{13}C HSQC spectra of the lignin isolated from the DES raffinates and 2-MTHF extracts in the aromatic region ($\delta_{\text{C}}/\delta_{\text{H}} = 98\text{--}132/5.8\text{--}8.2$ ppm). On top of the figures, the structures of the two main aromatic units are shown: syringyl (left, orange) and guaiacyl (right, blue). The peaks identified to these units in the spectra are labeled by color. Top left: lignin from the 1st 2-MTHF extract, Top right: lignin from the 2nd 2-MTHF extract, Bottom left: lignin from the 1st DES raffinate, Bottom right: lignin from the 2nd DES raffinate.

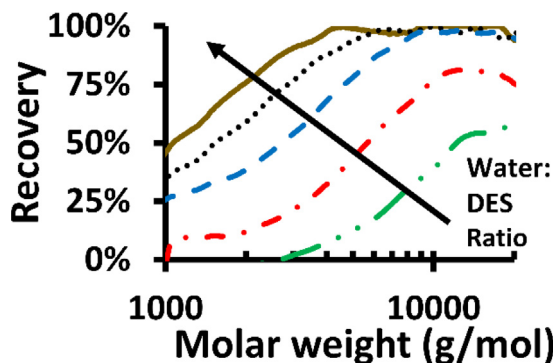


Fig. 6. Recovery of lignin by cold water precipitation using various DES to water mass ratios, 1:3.5 (solid), 1:1.7 (dot), 1:0.85 (dash), 1:0.42 (dash-dot), 1:0.22 (dash-dot-dot).

2000 g/mol could be removed, while for lignin fractions exceeding 10,000 g/mol, only 85% could be removed. Cold water precipitation could fully remove all lignin fractions above 4000 g/mol, while only 50% of the lignin around 1000 g/mol could be removed. In summary, recovery of the low molar weight lignin fractions is preferred by LLX, while recovery of the high molar weight fraction is preferred by cold water precipitation. Industrially, LLX is preferred over cold water precipitation since further removal of water from DES is highly energy intensive. However, a high (> 95%) lignin recovery is required for industrial applicability. In this study, the application of LLX was limited to DES with a water content equal or smaller than 25 wt%. In a previous study, we showed that the lignin distribution is highly dependent on the water concentration [27], and especially the distribution of the high molar weight fractions is affected by the water content. Therefore, we suggest LLX as the preferred method, with the addition of water to a total amount of 25 to 50 wt% based on the DES. In principle, the high molar weight lignin fractions can be precipitated by water addition prior or after LLX, but since the addition of water aids LLX, it is preferred to add water before LLX. Water can also be added during LLX to

aid the extraction of the high molar weight fractions [27]. The advantage of this method is that the number of unit operations is reduced, but precipitation before LLX produces a light and a heavy molar weight lignin fraction, which may bring more value to the produced lignins.

4. Conclusion

Cross-current extractions of lignin from a DES comprised of choline chloride and lactic acid were performed. It was found that 95% of the molar weight fraction around 2000 g/mol and 85% of the fractions above 10,000 g/mol could be recovered in 3 stages. Additional extraction stages were not performed, since the distribution coefficients dropped one order of magnitude from stage 1 to 3. Similar results were obtained when the lignin was directly extracted from the pulping DES, compared to the extraction of precipitated lignin that was re-dissolved in the DES. The lignin in DES was fractionated using a one stage extraction by 2-MTHF. Both fractions were analyzed by FT-IR and HSQC. It was found that the lignin that remained in the DES raffinate after two extractions did not contain any β - β or β -O-4 bonds. Together with the high molar weight of the lignin remaining in the DES, this suggests that the lignin remaining in the DES is highly condensed. Recovery of lignin by cold water precipitation could fully recover the lignin fractions heavier than 4000 g/mol, but only half of the lignin fraction around 1000 g/mol could be removed by the addition of 3.5 g water per g DES. For industrial applications, a combination of both approaches will be desirable due to their complementary nature of lignin recovery.

CRediT authorship contribution statement

Dion Smink: Investigation, Writing - original draft. **Sascha R.A. Kersten:** Supervision, Writing - review & editing. **Boelo Schuur:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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