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**DEEP EUTECTIC SOLVENT SYSTEMS FOR EFFECTIVE LIGNIN  
FRACTIONATION**

By

Gloria Agyapong

BSc. University of Cape Coast, 2020

MSc. University of Maine, 2024

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Chemical Engineering)

The Graduate School

The University of Maine

August 2024

Advisory Committee:

Sampath Gunukula, Professor of Chemical Engineering, Advisor

M. Clayton Wheeler, Professor of Chemical Engineering

G. Peter van Walsum, Professor of Chemical Engineering

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# **DEEP EUTECTIC SOLVENT SYSTEMS FOR EFFECTIVE LIGNIN FRACTIONATION**

By Gloria Agyapong

Thesis Advisor: Prof. Sampath Gunukula

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
Degree of Master of Science  
(in Chemical Engineering)  
August 2024

The carbon-rich biopolymer lignin has huge potential applications in producing bio-based products. However, the variability in lignin molecular weight, heterogeneity in lignin chemical structure, and limited functionality of lignin obtained from separation techniques prevent it from being widely used. In response to this challenge, a simple and eco-friendly approach was implemented to fractionate kraft lignin using deep eutectic solvents to produce homogenous lignin fractions with unique properties.

The following seven deep eutectic solvents were successfully made: choline chloride/ethylene glycol, choline chloride/levulinic acid, choline chloride/acetic acid, choline chloride/pyrocatechol, tetrabutylammonium chloride/ethylene glycol, tetrabutylammonium chloride/hexanol, and octanoic acid/hexanoic acid. Fourier Transform Infrared Spectroscopy (FTIR), and freezing point measurements were used to characterize Deep Eutectic Solvents (DESs), and the activity coefficient of each component in the mixture was calculated using the measured melting points. All seven DES mixtures showed significant temperature depression with negative deviations in the activity coefficient of each component at the eutectic point. These

deviations were used to derive general heuristics for screening potential hydrogen bond donors and hydrogen bond acceptors for DES formation and lignin fractionation.

Among the seven DESs developed, ChCl/Lev and TBACl-EG were used to fractionate kraft lignin. Kraft lignin is sequentially dissolved in ChCl/Lev, and TBACl-EG and stirred for 5 and 15minute respectively at 30°C. Different lignin fractions were obtained by gradual precipitation with water or acetone and centrifugation followed by vacuum filtration. Comprehensive analyses were conducted using thermal analysis (Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), functional group analysis (Nuclear Magnetic Resonance), and Elemental Analysis. The lignin fractions produced showed improved flexibility as compared to the parent kraft lignin with a Tg value reduction from 131°C to about 60°C revealed by DSC analysis. Improved thermal properties in lignin fractions was also observed between temperature ranges of 25°C to 200°C compared to the parent kraft lignin due to the rise in the specific heat capacities in the fractions. Elemental analysis revealed a reduction in carbon content with increased hydrogen content among the lignin fractions after fractionation. The overall thermal stability of lignin fractions over a temperature range of 100-800 was observed in the TGA and DTGA curves of the lignin fractions.

Thermogravimetric analysis (TGA) of the fractions obtained using ChCl-Lev DES revealed mass losses within the temperature range of 100-800°C as follows: the first fraction (F1), which dissolved after 15 minutes of extraction, exhibited a mass loss of 71%; the second fraction (F2), obtained after 30 minutes of extraction, showed a mass loss of 55.9%; the third fraction (F3), collected after 45 minutes of extraction, displayed a mass loss of 59.49%; and the undissolved fraction had a mass loss of 74.5%.

Similarly, for TBACl-EG DES, the weight loss percentages were observed as follows: the undissolved fraction experienced the highest weight loss at approximately 84%, followed by F1 at 77%, F3 at 70%, and F2 at 65%. Comparatively, kraft lignin exhibited a weight loss of approximately 52% at 800°C, retaining more weight due to its higher molecular weight compared to the fractions. Notably, among the fractions, F2 demonstrated greater thermal stability. Finally, proton NMR analysis on the lignin fractions revealed a variation in phenolic hydroxyl concentration and molecular weight, translating into a decrease in signal intensities amongst the lignin fractions.

## **DEDICATION**

I dedicate this thesis first and foremost to God Almighty for helping me through this journey. Next, to my brother, Moses Agyapong, and my parent Mrs. Owiredu Felicia and Mr. Kwadwo Agyapong for believing in me and supporting me in any way possible with constant prayers and encouragement. It has been my guiding light throughout this journey. Lastly, I would like to dedicate this work to George, for being there through it all and encouraging me through.

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## LIST OF ABBREVIATIONS

ASAM-	Alkaline-sulfite-anthraquinone-methanol
ATR-	Attenuated Total Reflectance
ChCl-	Choline Chloride
ChCl/Ac-	Choline Chloride/Acetic Acid
ChCl/EG-	Choline Chloride/Ethylene Glycol
ChCl/FA	Choline Chloride/Formic Acid
ChCl/Gly	Choline Chloride/Glycerol
ChCl/LA-	Choline Chloride/Lactic Acid
ChCl/Lev-	Choline Chloride/Levulinic Acid
ChCl/Py-	Choline Chloride/Pyrocatechol
ChCl/TEOA	Choline Chloride/Triethanolamine
ChCl/Urea	Choline Chloride/Urea
d-DMSO-	Deuterium Dimethyl Sulfoxide
DES-	Deep Eutectic Solvent
DI-	Deionized
DL-	Depolymerized lignin
DSC-	Differential Scanning Calorimeter
DTGA-	Derivative Thermogravimetry Analysis
EG-	Ethylene Glycol
FTIR-	Fourier Transform Infrared
HBA-	Hydrogen Bond Acceptor



HBD-	Hydrogen Bond Donor
HMWL-	High molecular weight lignin
KL-	Kraft lignin
LCB-	Lignocellulose Biomass
Lev-	Levulinic Acid
LMWL-	Low molecular weight lignin
LP-	Lignin precipitate
LR-	Lignin residue
MP-	Melting Point
NADES-	Natural Deep Eutectic Solvent
NMR-	Nuclear Magnetic Resonance
PY-	Pyrocatechol
TBACl-	Tetrabutyl Ammonium Chloride
TBACl/EG-	Tetrabutyl Ammonium Chloride/Ethylene Glycol
TBACl/Hex-	Tetrabutyl Ammonium Chloride/Hexanol
Tg-	Glass transition temperature
TGA-	Thermogravimetric Analysis
THEDES-	Therapeutic Deep Eutectic Solvent
TSE-	Twin Screw Extrusion
UV-	Ultraviolet

## CHAPTER 1

### INTRODUCTION

#### 1.1. Motivation

In recent times, there has been a significant surge in interest in integrated biorefineries because they play a vital role in moving toward a more sustainable and bio-based economy.<sup>1</sup> This is particularly important as it addresses challenges associated with the use of fossil resources including resource depletion and negative environmental impacts. Lignocellulose biomass comprising of carbohydrates (cellulose and hemicellulose) and lignin is therefore of interest as it serves as one of the most promising renewable feedstocks for biorefineries in producing biofuel, biochemicals, and biomaterials. Given the relative structural simplicity of the carbohydrates, biorefineries over the years have successfully converted these into valuable bio-products while lignin is mainly underutilized.<sup>2</sup>

Lignin is one of the most abundant polymers on earth surpassed only by cellulose. Depending on the source, lignin comprises about 20-30% of typical biomass. Its chemical structure is complex, highly heterogeneous, amorphous, and three-dimensional, composed of phenylpropane units obtained from three monolignols, namely p-coumaric alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S) illustrated in Figure 1.4 which are chemically linked by mainly either carbon-carbon, carbon-oxygen, and ether-ether bonds. The heterogeneity in the lignin structure is presented by random radical polymerization of the three different monomers during biosynthesis and fragmentation/condensation reactions during pretreatment and extraction processes.<sup>3</sup>

There are also variations in the structure of lignin. Depending on the plant species, extraction method, and operating conditions, different kinds of lignin exist that show different

levels of heterogeneity and lignin physicochemical properties. It is important to note that lignin's physicochemical properties impact its subsequent application. For instance, while high molecular-weight lignin characterized by high viscosity and carbon content is suited for making carbon fibers, low-molecular-weight lignin, known for high biological activity due to its highly reactive functional group is best suited for the polymer, pharmaceutical, and cosmetic industries. Lignin's high thermal stability and renewability make it a suitable material for biobased products. In addition, its growth expectations give it the advantage of being used as a feedstock for biobased products.

About 50 million tons of lignin is produced annually from the pulping process and the biorefineries globally. Only 2% is used for making dispersants and adhesives currently.<sup>4</sup> Lignin and its product market size has been reported by Rodrigues et al. (2021) to have an annual average growth rate of 2.28%, corresponding to US\$ 599 million in 2014 and expected to reach US\$ 704 million by 2022. It is essential, therefore, to optimize biomass utilization by fully exploring the potential of underutilized lignin to transition away from reliance on fossil resources and advance toward a sustainable and biobased economy. The simplest and most efficient way to reduce the structural heterogeneity of lignin is through lignin fractionation.<sup>5</sup>

A large and growing body of research has focused on improving the heterogeneity of lignin through three main approaches: gradient acid precipitation, membrane separation (ultrafiltration), and solvent fractionation. Solvent fractionation is by far the most efficient in obtaining lignin fractions with improved homogeneity and polydispersity.<sup>6</sup> Some recent studies have focused on investigating kraft/organosolv lignin molecular weight sorting employing the solvent fractionation approach mainly through three methods: Method 1) sequential dissolution in organic solvents with subsequent solvent evaporation, Method 2) dissolution in acetone with the gradual addition of

water for sequential lignin precipitation, and Method 3) dissolution in green solvents like gamma-valerolactone followed by ultrafiltration.

The first two approaches have seen success in improving the homogeneity of lignin and sorting lignin into low, medium, and high molecular weights.<sup>7</sup> Despite the potential for molecular sorting in the first two methods, there is a lack of efforts to establish fundamental relationships between organic solvent polarity, native lignin structure, functional groups, structural homogeneity, morphology, and resulting lignin fractions' molecular weights.<sup>7</sup> This limitation hinders the industrial application of lignin fractionation findings due to non-reproducibility observed in laboratory experiments.

Furthermore, in Method 2, the choice of antisolvent is arbitrary, and its effect on lignin molecular sorting is unknown and may require a large volume of antisolvent for precipitation which can translate into energy cost. In addition, Method 1 can be time-consuming, susceptible to error, and environmentally unfriendly.<sup>3</sup> Method 3 yielded contrary results as the lignin retained showed lower molecular weight than those in the filtrate. This unexpected result may be attributed to the extended configuration of lignin morphology in the presence of gamma valerolactone, as a molecular dynamic simulation study predicted. The simulation study indicated that lignin polymer attributes such as molecular weight are greatly influenced by solvent polarity. Hence, by adjusting solvent polarity during lignin fractionation, specific desired properties of lignin can be achieved.

Given that, the solvent polarity of Deep eutectic solvents (DESs) can easily be tuned between 0 and 1 by selecting appropriate combinations of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), It presents a promising choice for lignin fractionation and laying the correlation between solvent polarity, the native lignin structure, and the structural and polymer attributes of the desired lignin fraction. Deep eutectic solvents are solvents generally derived from

a combination of at least one hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) that is capable of associating through hydrogen bonding interactions when mixed at a fixed composition to present a eutectic mixture with temperature depression below the melting point of the pure components.<sup>8</sup> DESs are designer solvents that give some level of control over their properties by selecting appropriate combinations of their constituent.

Furthermore, DESs are considered biodegradable and environmentally friendly. It's important to note that numerous DESs can be synthesized from a vast possible combination of HBDs and HBAs, enhancing the versatility of this approach. However, the broad range of potential DES combinations requires a systematic screening approach to identify the most effective solvents for specific lignin properties currently lacking in the literature.

## **1.2. Aim and Scope of the Project**

In this study, the aim and scope include:

1. To synthesize simple and environmentally friendly deep eutectic solvents.
2. To deduce general heuristics to enable systematic screening of deep eutectic solvents for lignin fractionation.
3. To determine the stability of the deep eutectic solvents.
4. To demonstrate the use of the DESs for effective lignin fractionation.

## **1.3. Background**

### **1.3.1 Lignin Fractionation**

According, to Gigli and Crestini (2020) and Pang et al. (2021) lignin fractionation involves separating complex and varied lignin into several fractions, each with a narrower range of molecular sizes than the parent lignin with each fraction exhibiting distinct molecular weight distributions from one another as depicted in Figure 1.1.<sup>9</sup> To unlock the potential of lignin and

convert it into high-value products, extensive research has focused on reducing the structural heterogeneity of lignin through lignin fractionation. Studies indicate that the performance of lignin in specific applications is intricately linked to its molecular weight and polydispersity.<sup>10</sup> For instance, high molecular weight lignin with elevated carbon content and viscosity is better suited for carbon fiber production.

In contrast, low molecular weight lignin possesses enhanced antioxidant and antibacterial properties due to its abundant functional groups.<sup>11</sup> Lignin fractionation is done for two main purposes. The first is to examine the lignin's structure, primarily achieved through stepwise solvent extraction due to its efficiency in fractionation, ease of operation, and suitability for all types and sources of lignin. The second aim of lignin fractionation is to obtain lignin fractions with improved properties, promoting their application in various industries. All three methods of lignin fractionation have been utilized to obtain lignin fractions with enhanced properties, facilitating their upgraded utilization.<sup>9,10</sup>

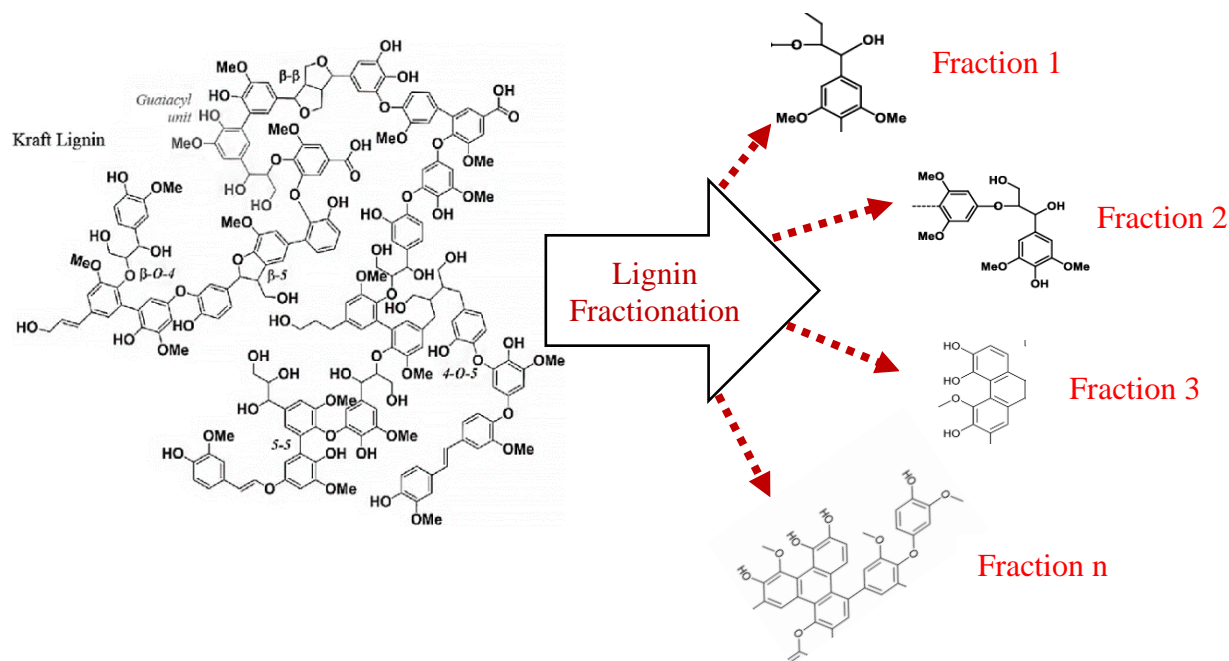


Figure 1. 1. The concept of lignin fractionation. Adapted from Park et al. (2018).<sup>6</sup>

### **1.3.2 Lignin Fractionation Approaches**

Various methods have been explored to reduce lignin heterogeneity. These approaches are categorized into gradient acid precipitation, membrane separation (ultrafiltration), and solvent-based fractionation.

#### **1.3.2.1 Gradient Acid Precipitation**

Acid precipitation is a commonly used technique to separate lignin from black liquor according to Figure 1.2.<sup>12</sup> Because of the negative charge on the colloid's surface, lignin is first dissolved in a basic solution to generate a stable colloid structure. When acid is added, the charge on the colloid surface is neutralized by H<sup>+</sup> ions, which causes the colloids to bond together and precipitate the lignin. It has been proposed that because low molecular weight lignin (LMWL) contains more negative groups, such as phenolic OH and COOH, the colloids it forms have a higher negative charge. Due to this property, LMWL colloids have a higher degree of colloidal stability than those made with HMWL (high molecular weight lignin).<sup>13</sup> Thus, high molecular weight lignin (HMWL) precipitates at higher pH values during the acid precipitation process of heterogeneous lignin, while low molecular weight lignin (LMWL) is recovered at lower pH levels.<sup>13</sup> As a result, precipitating lignin at progressively lower pH levels can be used to fractionate heterogeneous lignin. Gradient acid precipitation has been applied to lignin from a variety of sources, including alkaline-extracted lignin from steam-exploded corn stalks<sup>13</sup> pine Kraft black liquor<sup>14</sup>, oil palm trunk fiber pulping black liquor Sun and Tomkinson, (2001), and *Miscanthus sinensis* soda pulping black liquor.<sup>15</sup>

The goal is to obtain lignin fractions with narrow molecular weight distributions. The lignin fractions obtained at higher pH values have higher molecular weights than the fractions obtained at lower pH values. A range of inorganic acids, including phosphoric acid<sup>16</sup>, sulfuric acid<sup>14,15</sup>, and

hydrochloric acid<sup>13,17</sup>, have been employed sequentially to precipitate lignin from its alkaline solution. For example, alkaline-extracted lignin from steam-exploded corn stalks was precipitated using hydrochloric acid in a gradient acid process by Wang and Chen, (2013).<sup>13</sup> This procedure enables the selective fractionation of lignin according to molecular weight, whereby fractions with a greater molecular weight precipitate at pH values higher than those with a lower molecular weight at pH values lower. Wang and Chen, (2013b) performed the gradient acid precipitation of alkaline-extracted lignin from steam-exploded corn stalks using hydrochloric acid.

To precipitate the first fraction, the alkali solution that dissolved the parent lignin had to be acidified to a pH of 5.3. The second fraction was subsequently obtained by further acidifying the supernatant, which contained the unprecipitated lignin, to pH 4.0. The third fraction was created by acidifying the remaining soluble lignin at pH 4.0 to pH 2.0, and the final fraction was made up of the remaining soluble at pH 2.0. All four of the lignin fractions showed reduced polydispersity when compared to the parent lignin, and it was found that the molecular weight of the lignin fractions gradually dropped from the first to the last fraction. Moreover, the results of ultraviolet (UV) and FT-IR spectroscopy showed that the fractions of LMWL had the largest concentration of hydrophilic groups. Their improved solubility in water because of this property demonstrated the effective fractionation and modification of lignin using this gradient acid precipitation approach. For lignin solutions obtained from base separation techniques, such as soda and kraft pulping operations, gradient acid precipitation is primarily utilized with the use of this technology, most of the alkaline chemicals supplied during the lignin isolation process may be efficiently removed, resulting in samples of lignin solid that are relatively pure and have different molecular weights. This is beneficial for the lignin applications that come after. Nevertheless, this method's ability to effectively separate lignin fractions with varying molecular weights is typically



restricted, because at high precipitation pH values, LMWL is more likely to co-precipitate with HMWL. Consequently, some LMWL may still be present in the HMWL fraction even though the LMWL fraction can be retrieved at a low precipitation pH. As a result, the high molecular weight lignin fraction has a significant polydispersity.

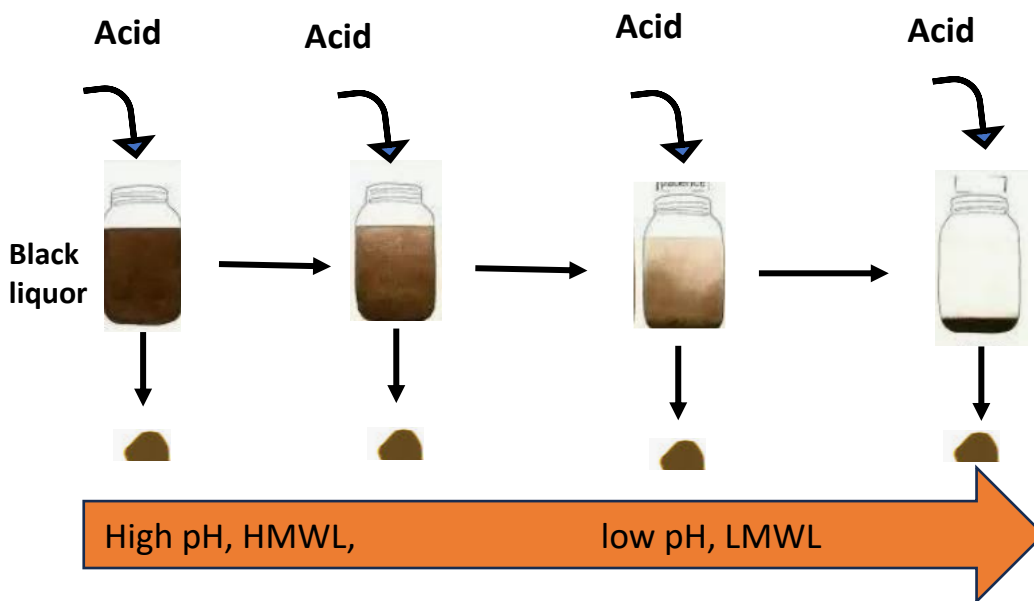


Figure 1. 2. Gradient acid precipitation process. Adapted from Pang et al. (2021).<sup>18</sup>

### 1.3.2.2 Membrane Separation

Fractionation by membrane separation has gained considerable interest due to the several advantages it provides over conventional solvent-based fractionation procedures which include its ability to be operated continuously, provide minimal energy consumption, no requirement for extra agents, and the ability to be combined with other methods.<sup>19,20</sup> The main tool used in membrane separation is a semipermeable membrane with different cut-offs. The semipermeable membrane serves as a selective barrier to divide two or more components according to their molecular weight.

They are mostly composed of ceramic or polymeric materials with unique properties that affect their effectiveness.<sup>21</sup> According to Baker, (2004), ceramic membranes are the most widely

used in industry settings because they provide a wider operating scope compared to polymeric membranes even though they require more harsh operating conditions (temperature, pH range, and longevity) and have less selectivity.<sup>22</sup> According to Fernandez-Rodríguez et al. (2018), ordinary ultrafiltration techniques are usually enough for effective lignin fractionation, as all lignin molecular weights lie within the range of ultrafiltration technology (100,000–1000 kDa). Large-scale lignin fractionation can be accomplished with ultrafiltration membranes with various molecular weight cut-offs by employing alkali-resistant and high-flux membranes.<sup>19</sup> For lignin treatment in membrane fractionation, two or three membranes with different cut-offs are usually used as shown in Figure 1.3. This is a very flexible approach that can be used directly on black liquor or to treat lignin alkali solutions such as black liquors from the alkaline pulping of *Miscanthus sinensis*<sup>23</sup>, black liquors from kraft pulping of birch, *E. globulus*, and spruce/pine combinations<sup>24,25</sup>, and black liquor from wheat straw/Sarkanda grass.<sup>26</sup>

In a study, Wang and Chen (2013) fractionated alkaline-extracted lignin from steam-exploded corn stalks using ultrafiltration membranes with varying cut-offs (6, 10, and 20 kDa).<sup>13</sup> A progressive drop in molecular weight was seen in each of the four lignin fractions obtained: 20 kDa retentate, 20 kDa permeate, 10 kDa permeate, and 6 kDa permeate. Furthermore, in comparison to the original lignin, all four fractions showed decreased molecular weight heterogeneity. Additional analysis of the chemical structures of the fractions showed that their chemical compositions were comparable, with an increase in acidic groups observed as the molecular weight dropped. Although lignin can be directly fractionated by ultrafiltration, a significant drawback is the solubility of most commercial lignin. According to Jaaskelainen et al. (2017), this frequently leads to membrane fouling, cleaning, decreased processing throughput, and eventually shortens the lifetime of the membrane in use.<sup>27</sup> To sum up, membrane fractionation

can efficiently extract inorganic salts and tiny molecular sugars from lignin solutions and enables exact lignin fractionation based on molecular weight. This approach is also distinguished by its affordability and convenience of use. Therefore, the ultrafiltration process has the most potential for application from an industrial standpoint.

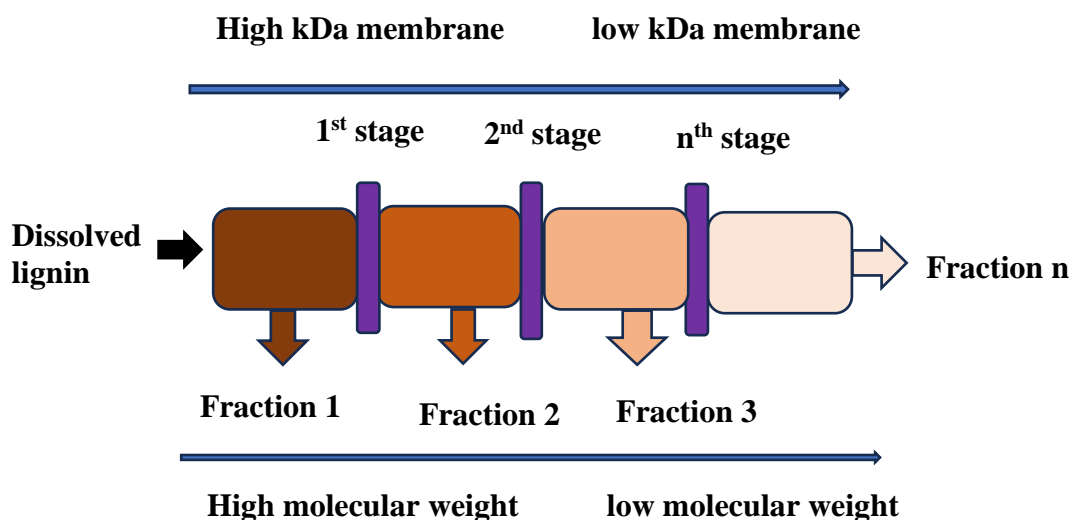


Figure 1. 3. Membrane filtration. Modified from Sadeghifar et al. (2020).<sup>10</sup>

### 1.3.2.1 Solvent-based fractionation

The genesis of solvent fractionation dates to the 1950s with Schuerch et al. where they showed that the Hildebrand solubility parameter and hydrogen bonding capacity of a solvent are the two main factors that determine the solubility of lignin in organic solvent. Organic solvent fractionation relies on the fact that lignin exhibits different solubility in diverse organic solvents due to variations in the molecular weights of the lignin fractions. In a broader context, lignin tends to be more soluble in solvents possessing a strong capacity for hydrogen bonding.<sup>28</sup> Hildebrand and Scott presented the solubility parameter, which is determined using the formula,  $\delta = \left( \frac{E}{V_m} \right)^{\frac{1}{2}}$

where;  $\delta$  is the solubility parameter, E is the solvent vaporization energy, and  $V_m$  is the molar

volume. According to this method, a solute has a higher probability of dissolving in a solvent whose Hildebrand solubility parameter ( $\delta$ ) is like its own. Schuerch, (1952) assessed a variety of solvents to see which ones worked best for dissolving lignin and discovered that solvents with  $\delta$  values of 22.5 MPa<sup>1/2</sup> worked best for this purpose. The statistics on  $\delta$  of lignin found in the literature typically fall between 28.0 and 29.9.<sup>29</sup>

A great deal of previous research into lignin fractionation using solvents has focused on three main approaches as illustrated in Figure 1.4. The first approach is the single-step organic solvent fractionation, the second is the sequential extraction using multiple organic solvents with water as an antisolvent, and the third is sequential precipitation using mixtures of organic solvents (one of the organic solvents acts as an antisolvent).<sup>30</sup> The single solvent approach is the most straightforward approach to fractionating lignin. The process generally involves utilizing a single organic solvent to subdivide lignin into two fractions: soluble and insoluble fractions. Typically, the soluble fractions exhibit a lower molecular weight compared to the original lignin, while the insoluble fractions display a higher molecular weight. The choice of solvent for this fractionation process should partially dissolve the technical lignin. The solubility of lignin in the solvent is influenced by factors such as the source of lignin and its structural characteristics, including molecular weight, functional group content, and monomeric composition.<sup>18</sup>

Various authors have assessed the efficacy of single solvent fractionation. Saito and colleagues highlighted the effective fractionation of softwood kraft lignin using methanol. This process resulted in methanol-soluble fractions that exhibited an elevation in low molecular weight and a decreased glass transition temperature.<sup>31</sup> Similarly, Li and co-workers used methanol as a solvent to dissolve Indulin AT Kraft softwood, Protobind 1000, and corn stover derived from cellulosic ethanol production. This process resulted in both methanol-soluble and methanol-insoluble

fractions. The portion of lignin that is soluble in methanol exhibited higher levels of aromatic hydroxyl groups and a greater S/G ratio, along with a lower molecular weight than the original lignin and the methanol-insoluble lignin fractions.<sup>32</sup> Even though single-step fractionation is simple, only two lignin fractions can be obtained with this fractionation method.

To achieve a narrower molecular weight distribution over two lignin fractions a simple sequential fractionation is required. Sequential fractionation aims to address the hurdles posed by single-step fractionation. The lignin's solubility in the selected solvents is enhanced by optimizing the ratio of the solvent combination. As a result, choosing the right organic solvents and combining them in the right amounts become crucial steps in the fractionation process. As such, fractionating lignin using mixtures of organic solvents turns into a complex process requiring a thorough understanding of both the chemistry of lignin and the properties of the solvent.

Currently, a range of blended organic solvents are used in this lignin separation process. Liu et al. (2017) applied a mixture of acetone and glycerol as well as ethanol and glycerol<sup>33</sup>, whereas Cui et al. (2014) employed a combination of acetone and hexane<sup>34</sup>, Jiang et al. (2017) used a combination of ethyl acetate and petroleum ether.<sup>35</sup> Liu et al. (2017) isolated lignin more successfully using enzymatic hydrolysis by varying the ethanol-to-glycerol ratio.<sup>33</sup> Elevating the ethanol content improved the high molecular weight fractions' solubility. This led to a decrease in phenolic acids and syringyl units but an increase in guaiacyl units, and an observed rise in molecular weight from 13,300 g/mol to 30,100 g/mol in three fractions.<sup>18</sup> Other solvents such as acetone, methanol, ethyl acetate, dichloromethane, and 2-butanone are identified in the literature for sequential extraction. Pang et al. (2021) noted that ethyl acetate and dichloromethane exhibited lower lignin solubility compared to the remaining solvents.<sup>18</sup> It is possible to perform successive fractionation of the primary lignin by the efficient use of several organic solvents.

To make the separation of low molecular weight lignin fractions easier, lignin's solubility in various organic solvents usually increases progressively. When choosing the right solvent, it is important to establish the solvent order based on the Flory-Huggins polymer-solvent interaction parameter ( $\chi_{PS}$ ). Because dimethyl sulfoxide has great lignin solubility, Passoni et al. (2016) suggest using parent lignin/dimethyl sulfoxide as a reference. Theoretically, if the  $\chi_{PS}$  value differs positively from this reference value, one could anticipate a less favorable solubility of the parent lignin in a solvent.<sup>1</sup> The use of multiple solvents increases cost, it is hard to control the solubility of the lignin in the solvent accurately. The solubility capacity of the mixture can be reduced if the ratio of the two solvents to be mixed is not well regulated. In addition, this approach requires a large volume of unsafe organic solvents.

Among organic solvents, acetone organosolv fractionation of lignin stands out as an energy-efficient, economical, and environmentally friendly approach. However, the use of hexane, a harmful chemical, should be replaced with a non-toxic solvent.<sup>36</sup> Liang et al. demonstrated that water, as a safe and environmentally friendly antisolvent, has a superior precipitation effect on lignin compared to methanol and dichloromethane.<sup>37</sup> However, this method increases the energy expenditure associated with water evaporation because it requires a large amount of water to precipitate lignin at the end of the fractionation process. Also, there is a lack of effort to establish fundamental relationships between organic solvent polarity, native lignin structure, functional groups, structural homogeneity, morphology, and resulting lignin fractions' molecular weights.<sup>7</sup> This limitation hinders the industrial application of lignin fractionation findings due to non-reproducibility observed in laboratory experiments. Furthermore, the choice of antisolvent used in precipitating lignin fraction is arbitrary, and its effect on lignin molecular sorting is unknown and may require a large volume of antisolvent for precipitation which can translate into energy cost.

The solvent fractionation process however offers a straightforward and efficient means to isolate lignin fractions possessing distinctive characteristics and it's by far, the most efficient in obtaining lignin fractions with improved homogeneity and polydispersity.<sup>6</sup> The limitations associated with solvent-based fractionation can be overcome by employing environmentally sustainable alternative solvents and solvents whose polarity can be easily tuned such as deep eutectic solvents for lignin fractionation.

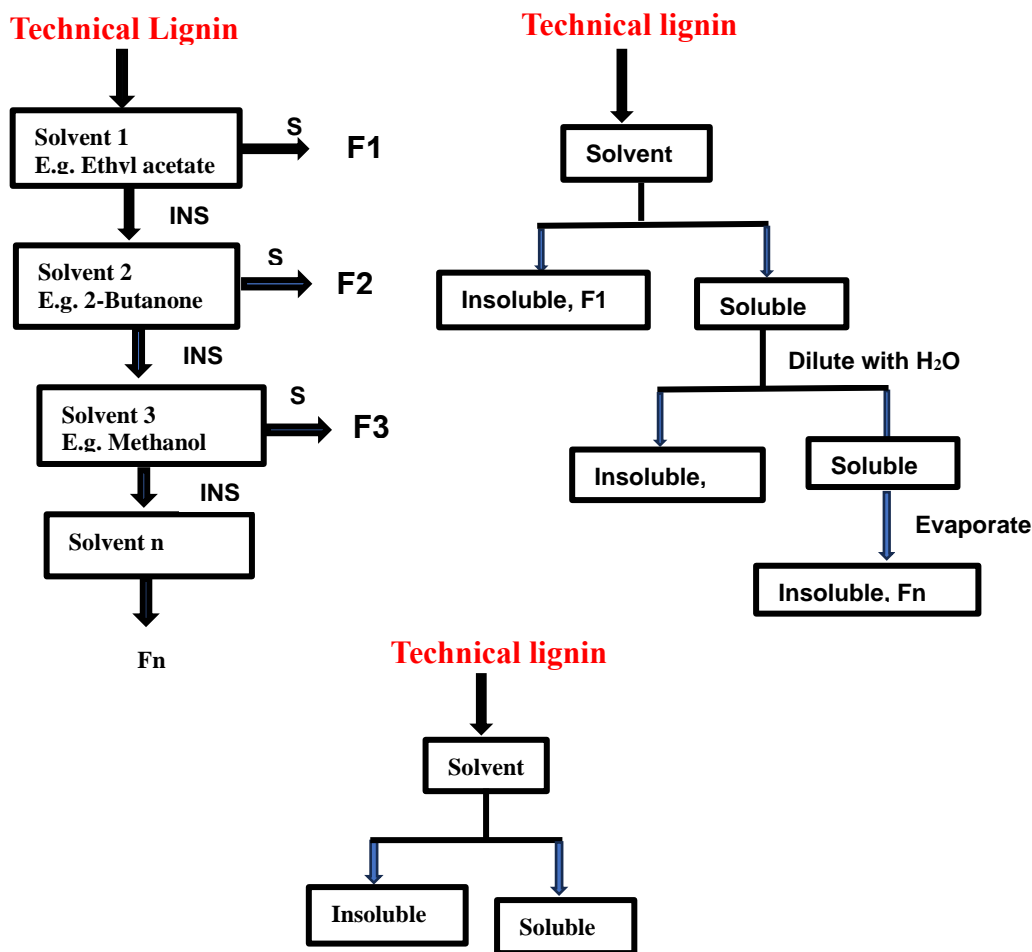


Figure 1. 4. Schematic illustration of the three general approaches employed for solvent fractionation. Modified from Sadeghifar et al. (2020).<sup>10</sup>

### 1.3.3 Deep Eutectic Solvents (DESs)

#### 1.3.3.1 DES Definition and Properties

Deep eutectic solvents (DESs) are a new class of solvents that have gained immense attention in recent years due to their enormous potential in many fields of study. DESs started 20 years ago as Deep Eutectic Mixtures (DEMs) when Abbott et al. (2001) were searching for liquids that could overcome moisture sensitivity and the high cost of some common ionic liquids.<sup>38</sup> Mixtures of different quaternary ammonium salts and metal salts were tested by these same authors and found that a mixture of choline chloride and zinc mixed at a molar ratio of 1:2 showed the lowest freezing point.

In the year 2003, these same authors researched eutectic mixtures of quaternary ammonium salts and hydrogen bond donors and referred to them as "deep eutectic solvents". The first of its kind was the choline chloride and urea 1:2 molar ratio mixture that presented a significant depression in freezing point temperature compared to that of the pure constituents. The depression in temperature was attributed to hydrogen bonding between the urea molecule and chloride ion and it was proven by NMR.<sup>39</sup> DESs are generally solvents made from a combination of two or three, (i.e., binary, or ternary) compounds consisting of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) capable of forming a eutectic mixture at a certain fixed composition through hydrogen bonding interactions.<sup>40</sup> Eutectic means melting easily, and a eutectic point is the point with the lowest melting temperature for any two solids at a fixed chemical composition.

The melting temperature should be lower compared to the melting point of the pure compounds involved. Several attempts have been made to define these solvents by different



researchers. However, a concrete definition for DES is still debatable because there several compounds that can associate through hydrogen bonding and have a eutectic point.<sup>41,42</sup> So, recently, Martins et al. came up with a definition that seeks to differentiate Deep eutectic solvents from other eutectic solvents being that "Deep eutectic solvents" are mixtures of two or more pure compounds with a eutectic point temperature below that of an ideal liquid mixture and deviating from ideality i.e., the difference between the ideal and the real eutectic point should be greater than zero.<sup>43</sup> In addition, the depression in temperature should be able to cause the mixture to stay liquid at operating temperature irrespective of the mixture composition. One of the key advantages of DES is their tunability. This means that their properties, such as viscosity, conductivity, and solubility, can be easily adjusted by varying the composition of the solvent. Other properties of DES are listed in Figure 1.5. This tunability allows researchers to optimize DESs for specific applications, enhancing their performance and efficiency. Moreover, Deep Eutectic Solvents are cost-effective and environmentally friendly. They are usually composed of a quaternary ammonium salt and a hydrogen bond donor, which are readily available and inexpensive compared to other solvents such as ionic liquids. Figure 1.12 demonstrates the formation of a deep eutectic mixture from two pure components A and B.

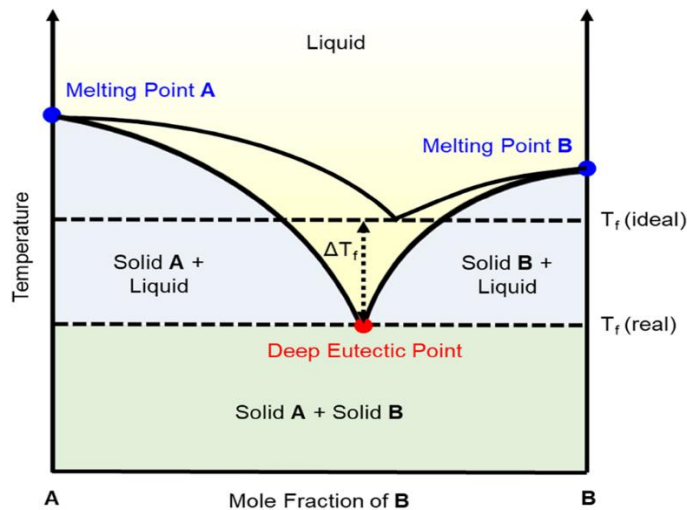


Figure 1. 5. Solid-liquid equilibrium diagram showing the formation of a DES.

Adapted from Han et al. (2023).<sup>44</sup>

### 1.3.4 Types of DES

Generally, most of the DES that have been reported in the literature can be categorized into five main classes, Type I, Type II, Type III, Type IV, and Type V based on the type of complexing agent used in their development as shown in Figure 1.6.<sup>45</sup> Type I DESs are developed from quaternary ammonium salts like choline chloride (ChCl), and metal halides ( $\text{AlCl}_3$ ).<sup>46</sup> A hydrated metal halide (such as  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ) plus a quaternary ammonium salt make up most of the Type II DESs. Due to their natural resistance to moisture and air, these DESs are better suited for industrial applications.

The Type III DESs are mixtures of a quaternary ammonium salt and other HBDs, such as carboxylic acids. The components of the Type IV DESs include inorganic transition metals and HBDs like urea. Type V DESs are often hydrophobic and consist only of non-ionic molecular materials.<sup>41</sup> It can be difficult to tell HBDs from HBAs in type V DESs. For the preparation of

type V DESs, it has been determined that the very strong interaction arising from the acidity differential between phenolic and aliphatic hydroxyl (OH) groups is essential.

Type V DESs often have increased volatility and are hydrophobic. They are superior to the other four DES types in that they provide the possibility of evaporation-based eutectic solvent renewal and recovery. This feature creates more practical pathways for the development of efficient DES recycling technology.<sup>41</sup> Since Type V DES was discovered so recently, no research has been done to determine how effective it is at lignin fractionation. Additional study is anticipated to broaden the application of type V DES in lignin fractionation, given its exceptionally effective lignin dissolution.<sup>47</sup> In addition, DESs can be categorized according to other attributes like pH levels and water miscibility. Based on their pH levels, they are usually divided into three classes: basic, neutral, and acidic DESs. The properties of the starting materials, in particular the functional groups found in the HBDs and acceptors HBAs, affect the pH of a given DES.

Furthermore, DESs can be divided into hydrophilic and hydrophobic groups according to how miscible they are with water. Few DESs are hydrophobic, and most DESs that are produced are hydrophilic. Hydrophobic DESs are often composed of components that are poorly soluble in water. They can be further classified into those that contain ionic compounds, such as tetra alkyl quaternary ammonium/phosphorous salts, and those that contain non-ionic compounds, such as monoterpenes.<sup>45</sup> A new category of DESs is represented by hydrophobic DESs. As a result, their potential for lignin fractionation is still largely untapped, requiring further research and work.

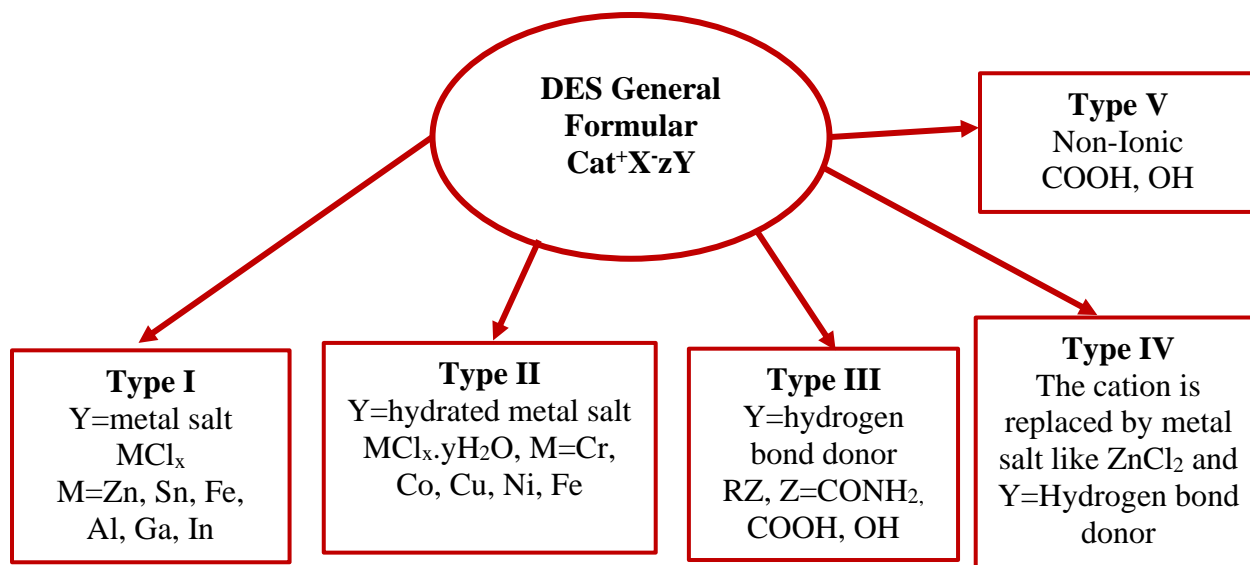


Figure 1. 6. General types of DES. Reproduced from Zhou et al. (2022).<sup>45</sup>

### 1.3.5 DES Preparation Methods

There are several methods used in literature to prepare deep eutectic solvents. The choice of method depends on the desired composition and properties of the DES. In general, there are about seven common methods used for preparing DESs: namely the heating and stirring method, free-drying method, vacuum evaporation method, grinding method, twin screw extrusion method, microwave irradiation method, and the ultrasound-assisted preparation method shown in Figure 1.7. Each method has its advantages and limitations, and the selection depends on factors such as cost-effectiveness, scalability, reaction time, desired properties of the final DES, equipment availability, the ability to minimize water, and personal preference. The two most widely used methods are the heating and stirring and grinding methods.

The heating and stirring method are the simplest and most used method. It involves mixing the desired hydrogen bond donor and acceptor in the desired ratio at room temperature or with mild heating, followed by stirring until a homogeneous mixture is obtained. The temperature

ranges from room temperature to moderately elevated temperatures, depending on the specific DES being prepared. Ruggeri et al. used a slightly different approach to this method. A water bath at 65°C used to heat the mixture for three hours containing the HBD and the HBA instead of heating directly on a hot plate after which the homogenous mixture was dried by either a vacuum oven, roto-evaporation, or in a desiccator.<sup>48</sup>

The advantage of this method is that it is straightforward to implement, requiring minimal equipment and resources.<sup>49</sup> However, the heating and stirring method may not be suitable for all DES compositions, as some components may require higher temperatures or special conditions to form a homogeneous mixture. Heating and stirring at a temperature that is too low or for heating and stirring at a shorter time led to the formation of crystals when a 1:2 mole ratio of choline chloride and ethylene glycol was prepared.<sup>50</sup> In addition, various authors reported damage to DES and the formation of by-products when prepared at elevated temperatures for a long time. It is therefore necessary to identify the appropriate temperature and stirring time needed to obtain a homogenous DES for all components in successful DES preparation. The freeze-drying method was revealed by Gutierrez et al. here, approximately 5 wt% of water is used to dissolve both components, separately. The two solutions are mixed frozen and subsequently free-dried to form a clear and homogeneous liquid.<sup>51</sup> In 2013, Dial et al. (2013) also reported the Vacuum evaporation method. In this method, the components are dissolved in water and dried under vacuum at 323 K to evaporate most of the water. The mixture is then kept in a desiccator with silica gel until a constant weight is reached<sup>52</sup>. Florinda et al. (2014) reported the grinding method. The method is suitable for two solid components. The two solid components are added to a mortar and ground until a clear, homogeneous liquid is formed.<sup>53</sup> In order to overcome the potential limitations of the heating and stirring method, Crawford et al. (2016) employed the twin screw extrusion (TSE)

method for large-scale preparation.<sup>54</sup> TSE is made up of two co-rotating or counter-rotating screws close-fitted in a stainless-steel barrel. The screw has multiple conveying and kneading sections. The conveying sections move the materials forward and the kneading section applies high shear and compression force on the material as it passes through. The HBD and the HBA constituents are manually mixed or mixed with a planetary ball mill when batch sizes are too large. Before the DES is preheated, all sections of TSE are preheated and the HBA and HBD are added in stoichiometric ratios through a feed port. At the other end of the TSE the colorless DES formed is collected and stored.<sup>54</sup>

To enhance the effective use of time and energy, Gomez et al. (2019) proposed a greener microwave-assisted method for preparing natural deep eutectic solvents within seconds<sup>55</sup> and Santana et al. (2019) reported the Ultrasound-assisted for preparing natural deep eutectic solvents<sup>56</sup>.

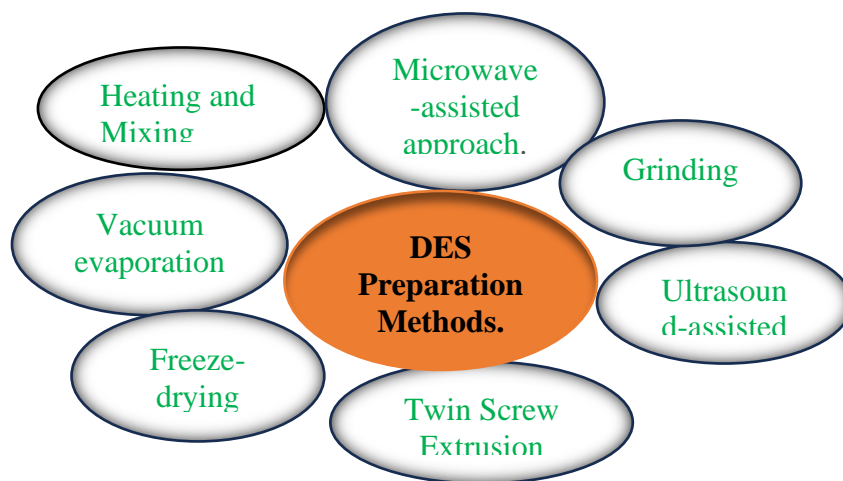


Figure 1. 7. The different methods employed in preparing DES.

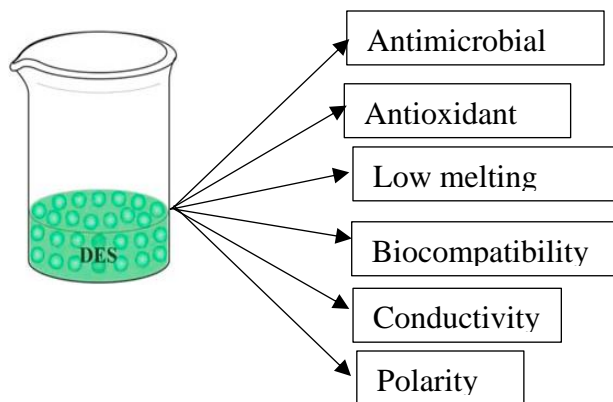


Figure 1. 8. Properties of DES.

### 1.3.6 Applications of DES

DESs offer a wide range of possible uses, and research on them is still being done in many different fields. This section attempts to demonstrate the potential and variety of applications that DESs offer, even though the applications covered here (Figure 1.8) are by no means all-inclusive. The research thrusts described below are a few of the most promising directions that have recently been investigated and reviewed by Hansen et al. (2021); Sharma et al. (2022); Shishov et al. (2017); Zhang et al. (2012)<sup>40,57-59</sup> which include metallurgy and electrodeposition, biomass processing, pharmaceuticals and medical research, nanomaterials synthesis, etc. Potential uses in metallurgy were among the first things that attracted attention to the DES class of solvents. Metals and metal salts were found to exhibit high solubilities and electrical conductivities in DESs, which made them attractive options for applications including electroplating/electrodeposition, resource refining, and metal extraction and recycling in solution. Utilizing techniques like recovery, purification, smelting, alloying, and plating, DES methods enable the creation of valuable stock materials from recycled and raw resources. This offers a quick start to metalworking and opens a variety of applications that use metals to make a variety of practical instruments.<sup>60</sup> When it comes to processing biomass, DES offers a viable substitute for traditional methods that are hazardous,

expensive, time-consuming, and require a lot of energy to transform biomass into valuable compounds.

As an alternative to conventional pretreatment, Kumar et al. (2016) investigated the solubility of rice straw lignin utilizing a variety of deep eutectic solvents made of "natural" chemicals (NADESs). Their results showed that the lignocellulosic biomass had solubilities of  $60 \pm 5\%$  (w/w) of total lignin, with extraction purities more than 90%. These and other investigations present novel possibilities for pretreatment methods.<sup>61</sup> Cheng et al (2023) investigated over 40 ChCl-based DESs ability to solubilize two industrial lignin (poplar and maize straw).

Effective lignin solubilization was demonstrated by pretreating poplar and maize straw with Choline Chloride/Formic Acid (ChCl/FA 1:3), Choline Chloride/Lactic Acid (ChCl/LA 1:3), Choline Chloride/Glycerol (ChCl/Gly 1:3), Choline Chloride/Ethylene Glycol (ChC/EG 1:3), Choline Chloride/Urea (ChC/Urea 1:3), and Choline Chloride/Triethanolamine (ChCl/TEOA 1:3). Of them, ChCl/FA (1:3) showed to be more effective in lignin solubilization, obtaining lignin solubilization rates of 82% and 57%, respectively, for both poplar and maize straw.<sup>62</sup> Garcia et al. (2015) reviewed the application of DES in CO<sub>2</sub>-capturing.<sup>63</sup> Mano et al. (2017) effectively synthesized gelatin membranes by electrospinning, utilizing a therapeutic deep-eutectic solvent (THEDES) consisting of mandelic acid and choline chloride in a 1:2 molar ratio. With optimal experimental settings, the gelatin solution contained 30% (w/v) gelatin and 2% (v/v) THEDES. These high-surface-area electro-spun fibers offer a wide range of uses. Most importantly, THEDES-containing gelatin fibers in PBS demonstrated a fast-release profile and minimal cytotoxicity, suggesting bright prospects for novel drug delivery methods.<sup>64</sup>

The centrifuge-less deep eutectic solvent-based magnetic nanofluid-linked air-agitated liquid-liquid microextraction method, an environmentally acceptable analytical technique for the



simultaneous preconcentration of lead, arsenic, cadmium, and copper, is presented in this study. With the use of DES-based carbon nanotubes and a special magnetic nano-fluid, this method makes separation simple and eliminates the need for centrifugation. Its efficacy in complicated matrices was demonstrated by showcasing its potential in a variety of samples, including walnut, rice, tomato paste, spinach, orange juice, black tea, and water.<sup>65</sup>

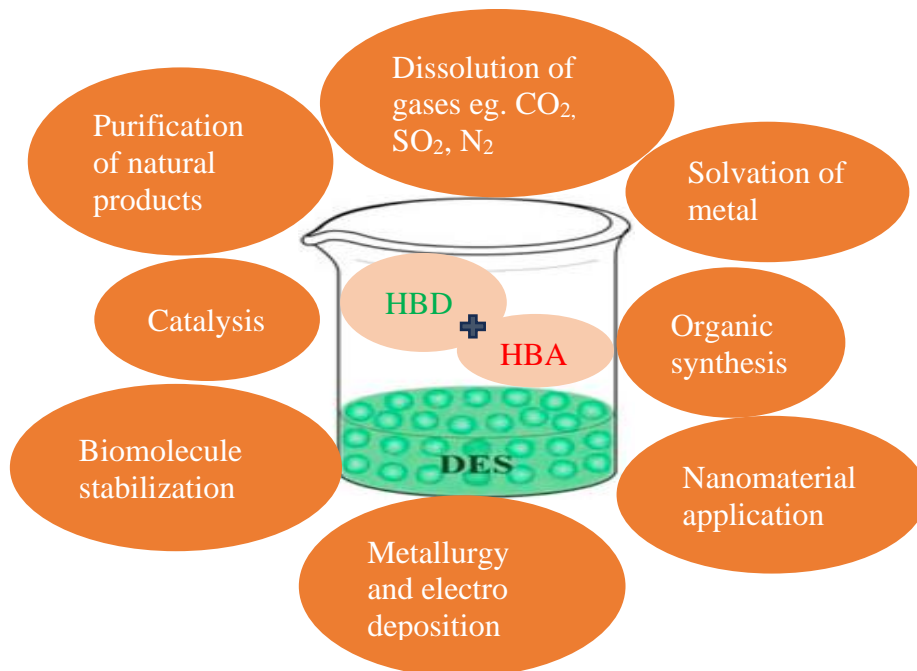


Figure 1. 9. Some applications of DES. Adapted from Singh et al. (2021).<sup>66</sup>

## CHAPTER 2

### METHODOLOGY

#### 2.1. Materials

Choline chloride and tetrabutylammonium chlorides were oven-dried at 100°C before use. All other materials were used without further purification. choline chloride (98% pure), tetrabutylammonium chloride (95% pure), pyrocatechol ( $\geq 99$  pure), hexanol ( $\geq 99$  pure), octanoic acid ( $\geq 99$  pure), and hexanoic acid ( $\geq 99$  pure) were obtained from Sigma-Aldrich. Ethylene glycol ( $\geq 99$  pure), levulinic acid ( $\geq 99$  pure), and p-Coumaric acid ( $\geq 98$  pure) were purchased from Acros Organic. Acetone (99.5%), vanillin, and acetic acid (99.7%) were also obtained from Fisher Chemicals. Kraft lignin (Indulin, AT) was purchased from Ingevity and was oven-dried for 8 hours at 100°C. Tetrabutylammonium chloride/Ethylene glycol (TBACl/EG) and choline chloride/levulinic acid (ChCl/Lev) DES were produced in our laboratory and stored at room temperature.

#### 2.2. Methods

##### 2.2.1 Preparation of DES

All DESs were synthesized via the most common heating approach. For each binary system, mixtures were prepared to cover the entire composition range. An analytical high-precision balance with an uncertainty of  $\pm 10^{-5}$ g was used to weigh the mass of each component into a cleaned vial with a cap. The mixture was heated to a temperature that is just below the boiling point of the two components for 1 h atmospheric pressure and stirred constantly until a clear homogeneous liquid was formed. The prepared DESs were cooled and stored at room temperature. The chemical names and abbreviations of the seven DESs prepared are shown in Table 2.1 below and the chemical structures of the HBA and HBD and the seven DESs prepared are shown in Figure. 2.1 and the structures in Appendix A.

Table 2. 1. The chemical structures of HBD and HBA are used in this work.

Component	DES Type	
HBA (A)	HBD (B)	
Choline Chloride	Ethylene Glycol	ChCl/EG
Choline Chloride	Levulinic acid	ChCl/Lev
Choline Chloride	Acetic acid	ChCl/Ac
Choline Chloride	Pyrocatechol	ChCl/Py
Tetrabutylammonium chloride	Ethylene Glycol	TBACl/EG
Tetrabutylammonium chloride	Hexanol	TBACl/Hex
Octanoic acid	Hexanoic acid	Oct/Hex

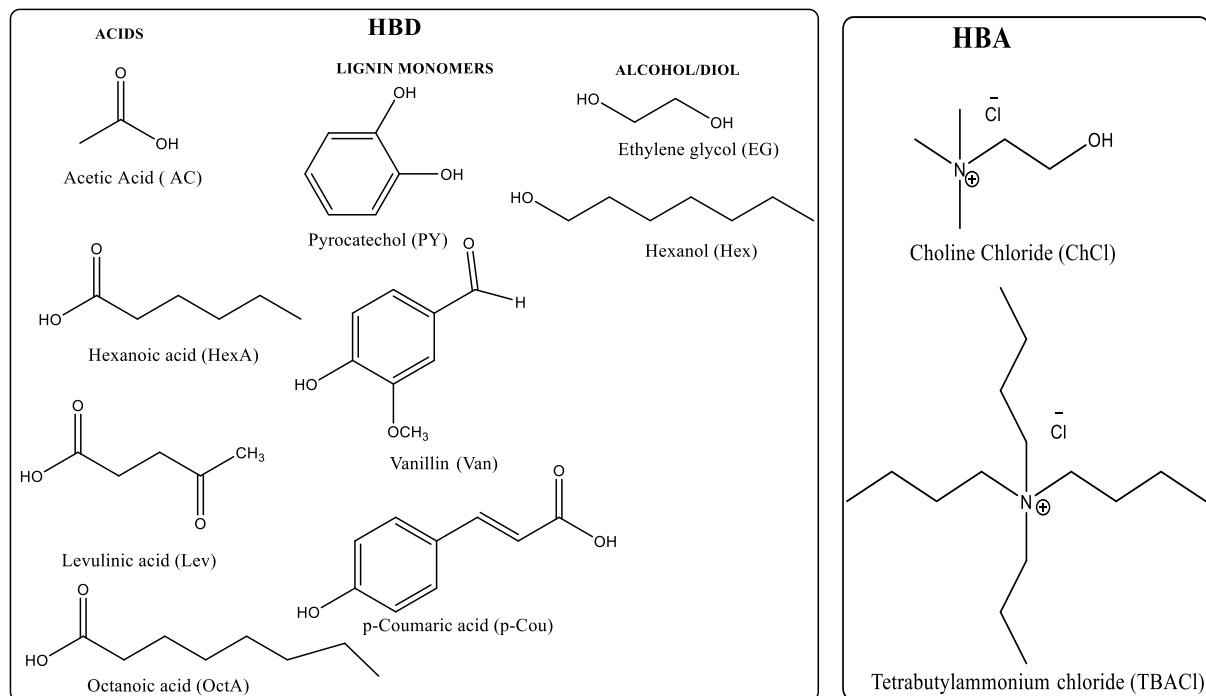


Figure 2. 1. The chemical structures of HBD and HBA used in this work.

## 2.2.2 Characterization of DES

### 2.2.2.1 Freezing Point Measurement

The freezing points of all the binary mixtures prepared at different molar compositions were measured using ethanol bath and dry ice. The vial containing the mixture samples is partially submerged into the ethanol bath with a k-type thermocouple thermometer, S220-T8, inserted into the sample for temperature measurement as shown in Figure 2.2. Dry ice is gradually added to the ethanol bath to decrease the temperature of the bath until the first crystal or solid is formed in the sample. The temperature at which the first solid is seen is recorded as the mixture's freezing point. The binary compositions of the binary mixtures that presented the lowest freezing point were selected as the Deep Eutectic solvent.



Figure 2. 2. Experimental setup for freezing point measurement.

### 2.2.2.2 FTIR Analysis

Fourier transform infrared spectroscopy was used to prove the existence of hydrogen bonding interactions between the two components in the binary mixtures at the deep eutectic point. A Nicolet i20 FTIR-ATR with a SMART iTX diamond ATR (Thermo Scientific) using 128 scans

per sample was used from 4000 to 600  $\text{cm}^{-1}$ . FTIR analysis was performed on the pure components and respective binary mixtures by placing a small amount of the sample on the surface of the diamond plate of the ATR.



Figure 2. 3. Nicolet i20 FTIR-ATR was used for this research.

### 2.2.2.3 Activity Coefficient Calculation

The activity coefficient of the components in the binary mixture is calculated to measure their deviation at the eutectic point using the Schroder-Le Chatelier equation 1 and the melting properties of the pure component listed in Table 3.2

$$\gamma_i = \frac{\exp\left[\frac{\Delta m h_i}{R} \left(\frac{1}{T_{mi}} - \frac{1}{T}\right)\right]}{x_i} \quad \text{Equation 1}$$

Where,  $\gamma_i$  represents the activity coefficient of component  $i$  in the mixture,  $\Delta m h_i$  and  $T_{mi}$ , are enthalpy of fusion and temperature of pure component  $i$  respectively, and  $T$  is the Freezing temperature of the mixture.

#### **2.2.2.4 DES Stability Test**

To test the stability of the DES when they are used for lignin fractionation, we conducted experiments with selected DES samples using lignin monomers (vanillin, p-coumaric acid, and pyrocatechol) as well as with kraft lignin. The aim was to investigate how adding a third component, which acts as a hydrogen bond donor, influences the DES structure. Various weight percentages of the lignin monomers were incorporated into the DES mixture and stirred continuously for an hour using a magnetic stirrer. Subsequently, the chemical structure of the mixture was analyzed using the Nicolet i20 FTIR-ATR instrument. The lignin monomers were added in percentages ranging from 10% to 100% by mass. The stability of the DESs were also tested with acetone a potential antisolvent to study its interaction with the DES.

#### **2.2.2.5 Lignin Solubility in DES**

The solubility of Lignin in DES was measured by saturating the DES with lignin at 30°C and atmospheric pressure. The saturation point of the different DES was determined by adding Kraft lignin and stirring under constant stirring with a magnetic stirrer at 30°C until the DES reached saturation. Initially, 1% of the mass of the lignin is added to the DES and stirred until complete dissolution is observed. If the lignin has dissolved, approximately 1% more is added to the mixture and stirred. The procedure was repeated until solids were observed remaining in the solution, with the previous mass recorded as the solubility. The results are presented in Table 3.3.

### **2.2.3 Lignin Fractionation method**

#### **2.2.3.1 Sequential Lignin Fractionation using Single DES (TBACI/EG, ChCl/Lev DES)**

One gram of kraft lignin is partially dissolved in 10g of the DES in a vial, stirred at 30°C, and filtered through vacuum filtration for dissolved and undissolved fractions. The undissolved

fraction is redissolved into a fresh batch of the same DES and the approach is repeated twice for the second (F2) and third fraction (F3) plus the undissolved fraction. The same approach is employed for ChCl/Lev DES fractionation. Stirring was done for 15 and 5 minutes for ChCl/Lev and TBACl/EG DES fractionation respectively. The ratio of lignin to DES was always maintained at 1:10. Two antisolvents (DI water and acetone) were employed to precipitate dissolved lignin. After precipitation, the mixture is centrifuged at 2000 rpm for 5 minutes before vacuum filtering. Acetone-precipitated lignin fractions were air-dried for 24hrs, and DI water fractions were air-dried for 48hrs before characterization.

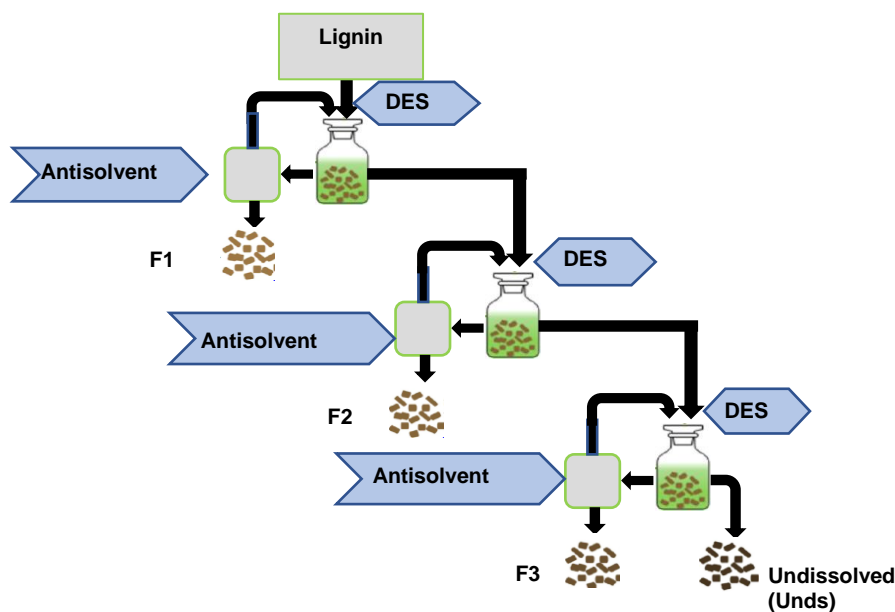


Figure 2. 4. Lignin fractionation approach employed.

### 2.2.3.2 Sequential Lignin Fractionation using Multiple DES.

One gram of kraft lignin is first partially dissolved in 10g TBACl/EG DES and stirred constantly with a magnetic stirrer at 30°C for 5min. The mixture is vacuum filtered to obtain two fractions, TBACl/EG soluble fraction and the undissolved fraction. The undissolved fraction is

redissolved into ChCl/Lev DES and stirred for 15min. The mixture is vacuum filtered again for the ChCl/Lev soluble fraction and the undissolved fraction. The ratio of lignin to DES was always maintained at 1:10. The TBACl/EG soluble and the ChCl/Lev DES soluble lignin fraction was precipitated using DI water. After precipitation, the mixture is centrifuged at 2000 rpm for 5 minutes before vacuum filtering. The fractions are air-dried for 48hrs before characterization.

## **2.2.4 Lignin fractions Characterization Techniques**

### **2.2.4.1 DSC**

The glass transition temperature of the lignin fractions was measured using the Differential Scanning Calorimetry. A TG, DSC-2500 apparatus was used to perform differential scanning calorimetry (DSC) at a flow rate of 20 milliliters per minute in a nitrogen atmosphere. In this experiment, samples (about 5-15 mg) were heated at a rate of 10 °C/min, with a temperature range of 25°C to 250 °C.

### **2.2.4.1 TGA**

Thermogravimetric Analyzer, Q500 was used to further characterize the obtained lignin fractions, samples of about 15 mg were loaded onto the platinum trays and placed onto the sample holder via the autosampler. The furnace was then lifted over the sample and the temperature ramp was started. The method employed was a temperature increase from 10°C/min from room temperature to 900°C while the mass loss was studied. Show a sample of the TGA graph and explain.





Figure 2. 5. The TGA Q500 used to study the mass loss of the parent kraft lignin and the obtained lignin fractions.

#### **2.2.4.2 H-NMR**

Proton NMR analysis was carried out on all obtained lignin fractions and the parent lignin. The Bruker AVANCE 400 spectrometer operated below room temperature with 128 scans were used. Approximately 50mg of the sample is added to 0.6 mL deuterium Dimethyl sulfoxide (DMSO-d<sub>6</sub>) in a 5mm NMR tube. The sample is allowed to dissolve in the solvent before running the analysis.

#### **2.2.4.3 Elemental Analysis**

An elemental analyzer, flash 2000 CHNS/O was used to analyze the elemental compositions of the obtained lignin fraction. For each fraction, the CHN composition was measured three times for three different fractionations.



Figure 2. 6. Elemental analyzer used in this work.

## CHAPTER 3

### RESULTS AND DISCUSSION

#### 3.1. Assessing the Formation of DES

All DES were synthesized using the most common and simple method of synthesis, the heating method. The purity of the DES was not assessed. Table 3.1 depicts the results of all DES prepared. It can be observed from the table that not all combinations of an HBD and HBA yielded a DES. For example, when Choline chloride was combined with vanillin in three different ratios (1:1, 2:3, and 1:2), they formed Deep Eutectic Solvents (DES) under the synthesis condition. However, these mixtures solidified quickly once cooled to room temperature, with the 1:1 ratio solidifying after about 24 hours. This indicates that a strong hydrogen bond was present between choline chloride and vanillin during synthesis at 80°C but weakened as the temperature decreased to room temperature.<sup>67</sup> Choline chloride and p-coumaric acid did not form a DES even when different molar ratios of the compounds were tested at 80°C. According to Kim et al. (2018), a combination of Choline chloride with p-coumaric acid forms a DES at 100°C and solidifies after 2-3 days.<sup>67</sup>

Furthermore, when Tetra butyl ammonium chloride is mixed with vanillin in a 1:1 molar ratio, it forms a viscous liquid at room temperature. However, it does not form DES with p-coumaric acid, regardless of the ratios used. This observation suggests that Tetra butyl ammonium chloride can interact with vanillin at 80°C but not with p-coumaric acid. Again, combinations of choline chloride and hexanol did not form a DES, white precipitates of the choline chloride in hexanol were observed indicating that there was no interaction between the two components when mixed. Overall, these results indicate that the success of forming a DES depends on many factors which include hydrogen bond strength of the components, stoichiometry, miscibility of the

components, and functional group compatibility. Currently, no general heuristics in the literature guide the selection of potential DES-forming compounds given the wide range of possible combinations. That is why one of the goals of this research section is to develop general heuristics that can be utilized in solvation chemistry simulations to screen potential components for forming DES and are effective in lignin fractionation. The following sections describe three general heuristics developed in this work.

Table 3. 1. Summary of different DESs Studied in This work using Choline Chloride and Tetra butyl ammonium chloride as the HBA and alcohols, diols, acid, and lignin monomers as HBD together with their eutectic compositions.

DES Type	HBA (A)	HBD (B)	Eutectic Composition of HBA: HBD	State at room Temperature.
ChCl/EG	Choline Chloride	Ethylene Glycol	1:4	Clear liquid
ChCl/Lev		Levulinic acid	1:2	Clear liquid
ChCl/Ac		Acetic acid	1:2	Clear liquid
ChCl/Py		Pyrocatechol	1:1	Viscous liquid
TBACl/EG	Tetrabutylammonium chloride	Ethylene Glycol	$x_A \leq 0.2$	Clear liquid
TBACl/Hex		Hexanol	$x_A \leq 0.2$	Clear liquid

Table 3.1 continued

Oct/Hex	Octanoic acid	Hexanoic acid	1:4	Clear liquid
Table 3.1 continued				
ChCl-Van	Choline Chloride	Vanillin	-	Solid
ChCl-p-Cou	Choline Chloride	p-Coumaric acid	-	Solid
TBACl-Py	Tetrabutylammonium chloride	Pyrocatechol	-	Solid
ChCl-Hex	Choline Chloride	Hexanol	-	White solid ppt
TBACl-Van		Van	1:1	Highly viscous liquid

### 3.2. Assessing the formation of DES using FTIR measurement.

The FTIR-ATR is used to study the interaction between different components, analyze and identify structures of the prepared mixture, and to confirm the formation of DES. Focus was placed on the chemical structure of the seven prepared DESs (Figure 3.1b) studied and compared to that of its corresponding HBDs Figure 3.1a.

For the seven DESs prepared, the FTIR spectra showed a broadened peak at 3500-3000  $\text{cm}^{-1}$  in the hydroxyl group (-OH) stretches of the DES as compared to their respective HBD. The broadened peak is due to the stretching vibrations of the hydroxyl group due to association as compared to the free hydroxyl group present in the pure component. These results indicate that DESs were successfully prepared. Moreover, the DES spectra clearly show distinctive absorbances like the C-N of choline chloride (about 955  $\text{cm}^{-1}$ ).<sup>62</sup>

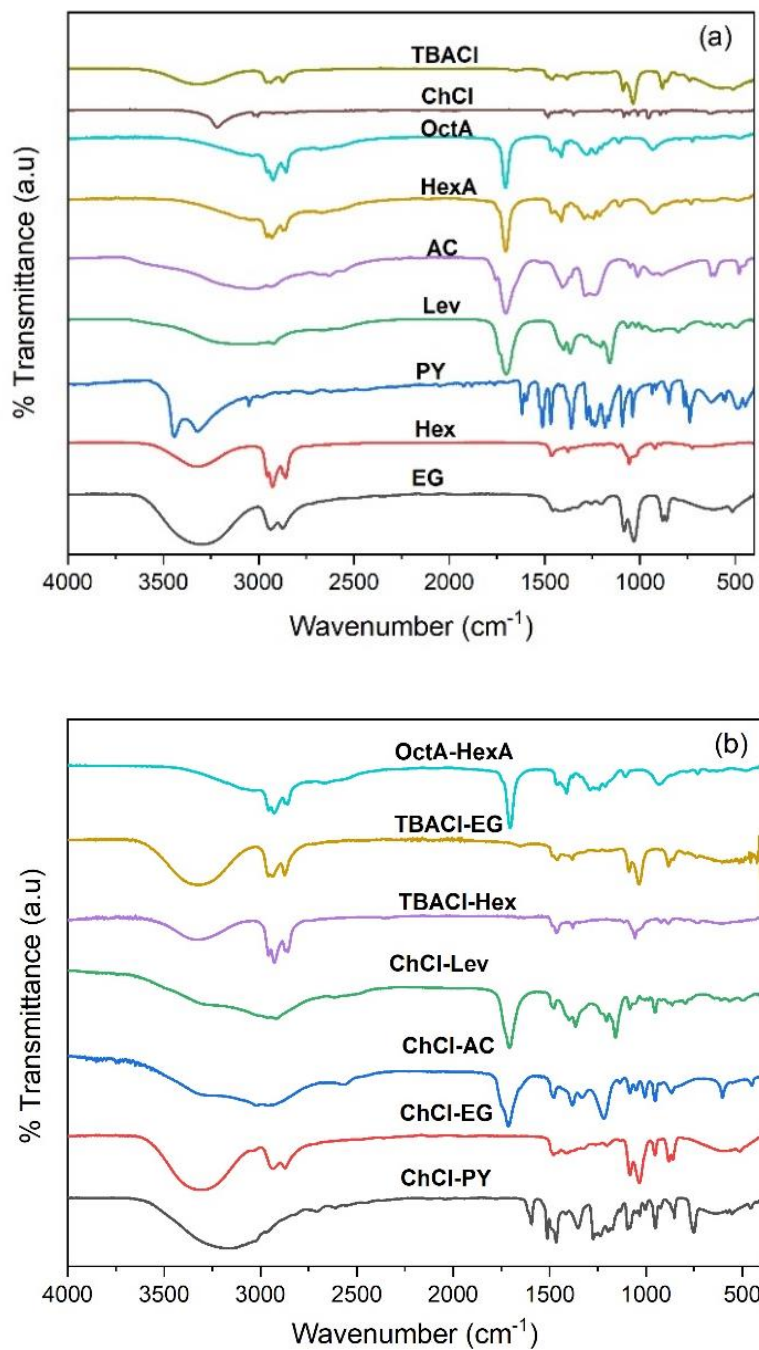


Figure 3. 1. FTIR spectra of HBD, HBA (a), and DES (b).

### **3.3. Assessing the formation of DES by freezing point measurement.**

The freezing points of all the seven binary mixtures developed were measured across a wide range of molar compositions to identify the eutectic composition, which represents the composition with the lowest freezing point. The eutectic composition and freezing point of the seven developed DES are summarized in Table 3.2 below. It can be observed from the table that the freezing point of all the developed DESs were far below the freezing point of their pure components. This suggests that a stronger hydrogen bonding interaction exists between the two components relative to the intermolecular interactions existing in the liquid phase of the pure components.<sup>41</sup> This depression is also illustrated in the phase diagrams for solid-liquid equilibrium presented in Figures 3.2, 3.3, and 3.4.

Table 3. 2. Summary of different DESs studied in this work using choline chloride and Tetra butyl ammonium chloride as the HBA and alcohols, diols, acid, and lignin monomers as HBD together with their eutectic compositions, activity coefficient, enthalpy of fusion ( $\Delta H_m$ ) of the pure components, and the measured freezing points of the developed DES.

Component		Eutectic Composition Of HBD: HBA	$\Delta H_m/KJ/mol$		Melting point of pure components		Measure d melting point of mixture ( $^{\circ}C$ )	Activity Coefficient	
HBA (A)	HBD (B)		A	B	A	B		$\gamma_A$	$\gamma_B$
Choline Chloride	Ethylene Glycol	1:4	4.3	11.2	324	-12.9	-64	<1	<1
Choline Chloride	Levulinic acid	1:2		9.22	324	33	-52	<1	<1
Choline Chloride	Acetic acid	1:2		11.72	324	16.6	-51	<1	<1
Choline Chloride	Pyrocatechol	1:1		22.87	324	100-103	-8.2	<1	<1
Tetrabutylammonium chloride	Ethylene Glycol	$x_A \leq 0.2$	14.69	11.2	70	-12.9	<-72	<1	<1
Tetrabutylammonium chloride	Hexanol	$x_A \leq 0.2$		15.48	70	-47	<-61	<1	<1

### 3.4. General Heuristic I (Activity Coefficient at the Eutectic point)

When components interact within a system, their behavior deviates from their behavior in isolation. The activity coefficient quantifies this behavior by measuring the extent of deviation from ideal behavior in solutions.<sup>68</sup> One notable characteristic of DESs is the significant freezing point depression upon mixing components, particularly pronounced at the eutectic composition. However, there is no consensus on the extent of this decrease necessary to classify a specific eutectic mixture as a "deep eutectic solvent," nor is there agreement on the appropriate reference



point for this classification. To account for this, the activity coefficient of each component in the binary mixtures was calculated using Equation 1. These calculations were based on both the melting properties of the pure components and the measured temperatures. The results were then compared to the predicted solid-liquid phase diagram of the mixtures, which was derived from Equation 1 using the melting properties of the pure components.

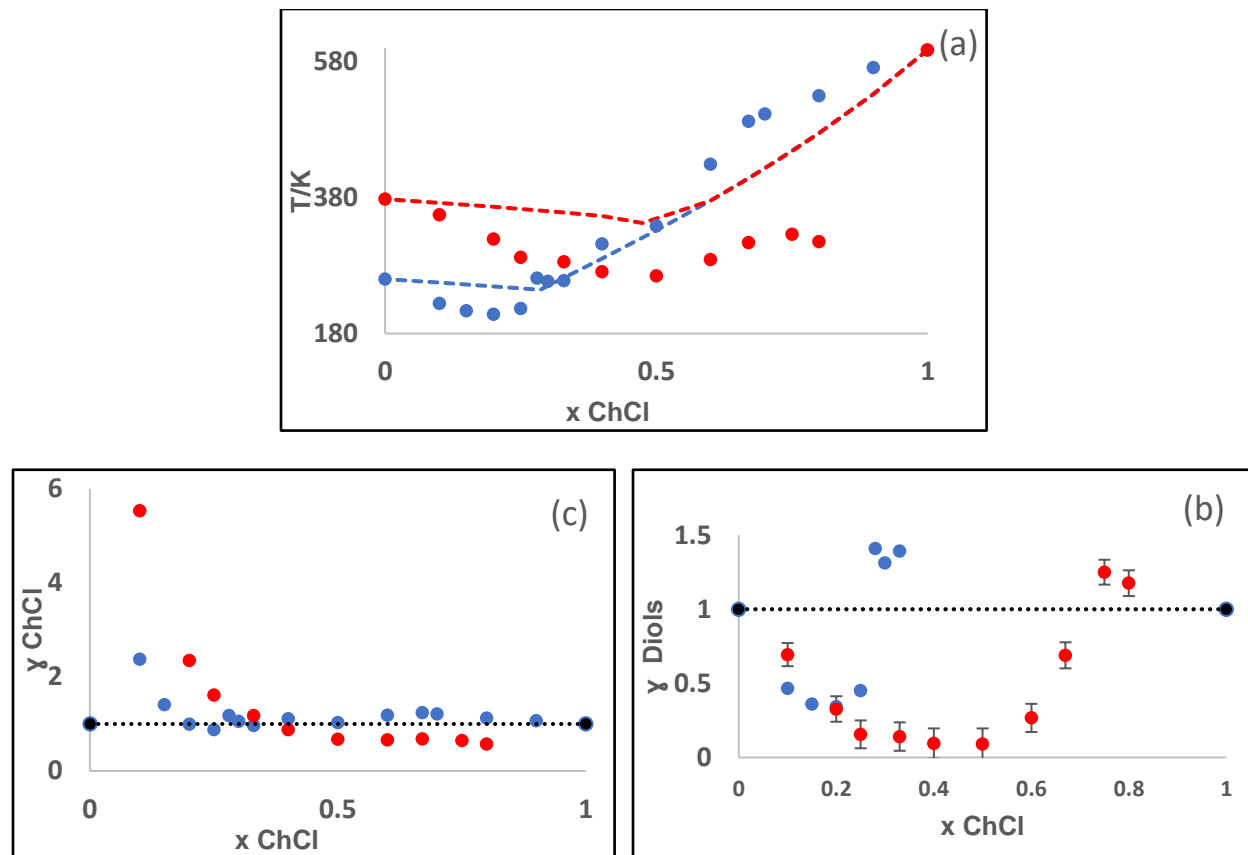


Figure 3. 2.Solid-liquid phase diagram (a) and corresponding activity coefficient of the Diols (b) and Choline chloride (c) for the systems composed of choline chloride and ethylene glycol (blue dot), Choline chloride Pyrocatechol (red dot). Dotted lines represent thermodynamic ideality ( $\gamma = 1$ )

It can be observed from Figure 3.2 that both diols and choline chloride exhibited negative deviation negatively thermodynamic ideality when mixed, which are caused by the diols. In the

case of ethylene glycol, a negative deviation is induced at a lower concentration of choline chloride, resulting in the formation of a deep eutectic solvent. Similarly, for all the compositions of choline chloride pyrocatechol mixtures, pyrocatechol induces negative deviations from ideality leading to the formation of deep eutectic solvents. The formation of Deep eutectic solvent from the combinations of choline chloride and the two diols is because the molecular interactions established between the mixtures at lower concentrations of choline chloride are stronger than the interactions present in the liquid phase of the pure compounds.

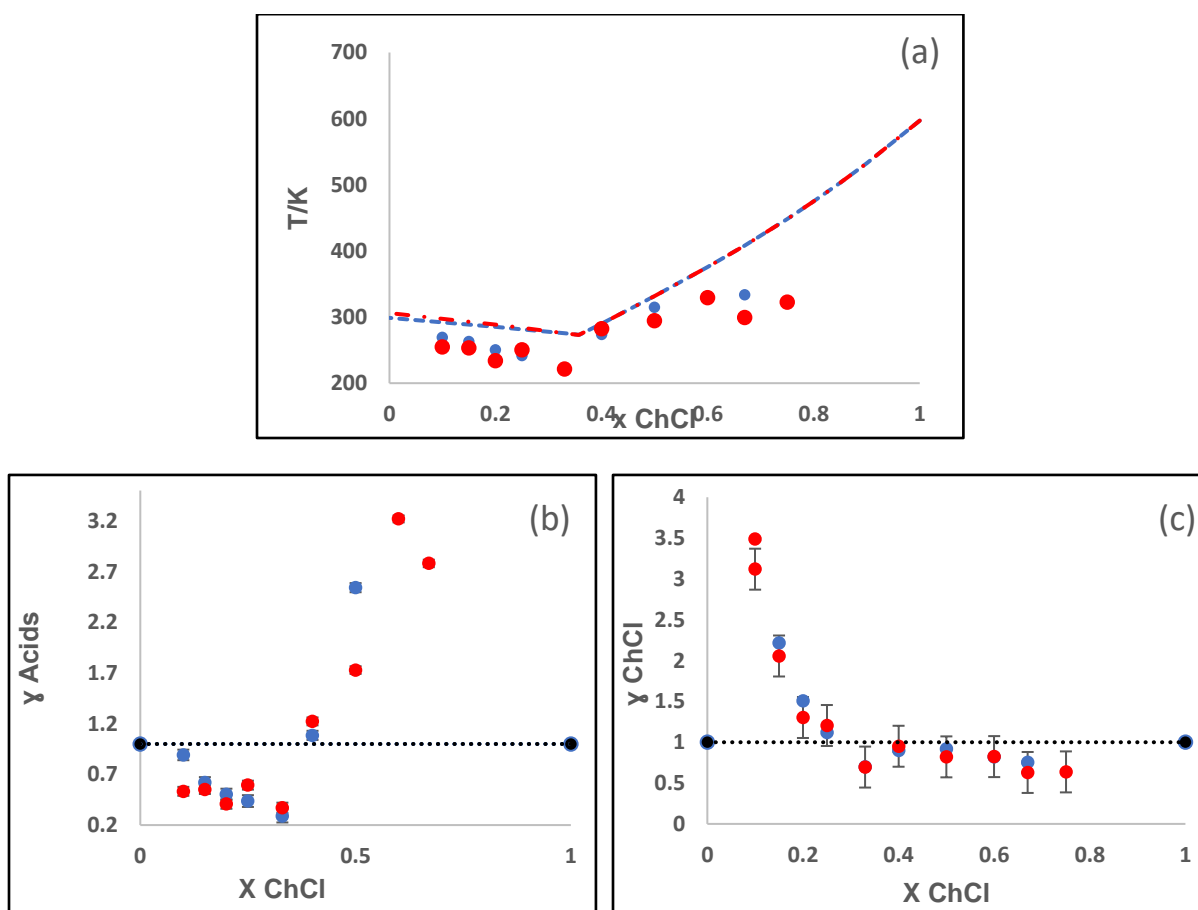


Figure 3.3. Solid-liquid phase diagram (a) and corresponding activity coefficient of the Acids (b) and Choline chloride (c) for the systems composed of choline chloride and acetic acid (blue dot), Choline chloride and levulinic acid (red dot). The dotted lines represent thermodynamic ideality ( $\gamma = 1$ ).

Like choline chloride/diol systems, the acids (levulinic acid and acetic acid) were able to induce negative deviations from thermodynamic ideality when mixed with lower concentrations of choline chloride to form a deep eutectic solvent. The molecular interaction existing between this system is again stronger than the one existing between the liquid phase of the pure components (Figure 3.3).

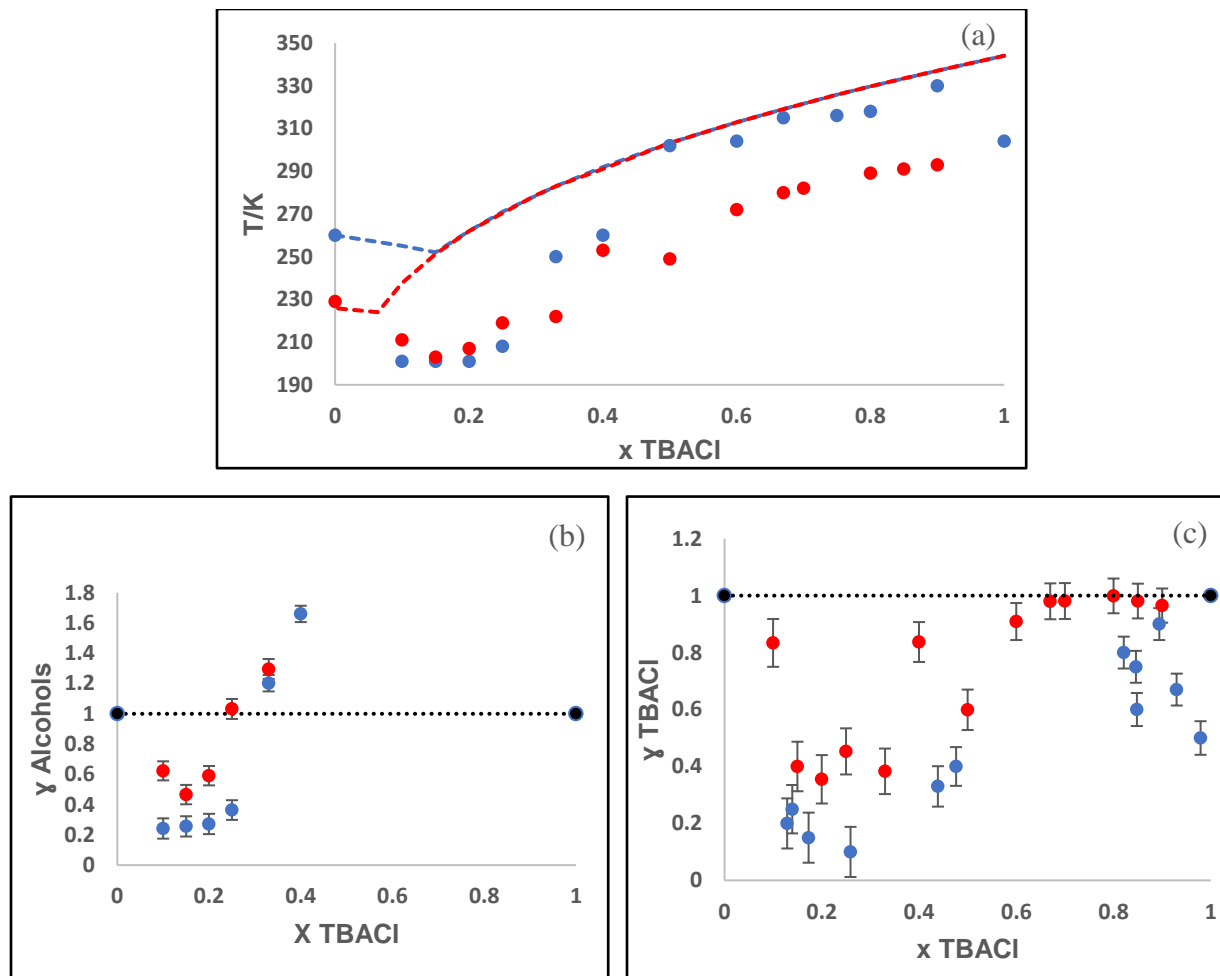


Figure 3. 4. Solid-liquid phase diagram (a) and corresponding activity coefficient of the Alcohols (b) and Tetra butyl ammonium Chloride (c) for the systems composed of Tetra butyl ammonium Chloride and Ethylene glycol (blue dot), Tetra butyl ammonium Chloride and hexanol (red dot). Dotted lines represent thermodynamic ideality ( $\gamma = 1$ )

Figure 3.4. demonstrates the usefulness of Tetra butyl ammonium chloride in forming deep eutectic solvents. The tetra butyl ammonium chloride/alcohol system formed deep eutectic solvents when mixed with lower concentrations of tetra butyl ammonium chloride. Negative deviations to ideality are observed in both the alcohols and tetra butyl ammonium chloride suggesting that stronger intermolecular interactions exist in the mixtures compared to the interactions existing in the pure component liquid phase. It is important to note that tetra butyl ammonium chloride presented higher negative deviations to ideality at different composition ranges when combined with ethylene glycol than choline chloride. Observations from solid-liquid equilibrium diagrams and activity coefficient plots reveal that at the eutectic point, the activity coefficients of all components within the developed DES exhibit negative deviations.

This discovery implies that for any deep eutectic solvent, the constituents involved in its formation have a negative deviation from ideality at the eutectic point. This conclusion aligns with the findings of Abranches et al. (2020). Further, there is a need to quantify the degree of negative deviation from thermodynamic ideality at the eutectic point required for each component and assess how this deviation impacts the ability of the solvent to dissolve lignin. This quantification is essential for the effective screening of DES for lignin fractionation.

### **3.5. General Heuristic II (Activity Coefficient and Lignin Solubility).**

Table 3. 3. compares the activity coefficient of each component of the DES developed at the eutectic point and the solubility of lignin in the DES. From the table, it can be observed that the solubility of lignin in the DES is minimal at greater negative deviations in either of the components (HBD or HBA) in the mixture. While the reason for this result is unclear, several factors could be contributing to it. Potential explanations could include the level of hydrogen bond

strength existing between the two components. Although strong hydrogen bonding can lead to desirable properties such as stability, it can also lead to challenges such as reduced solubility due to an increase in the solvent's viscosity. Therefore, understanding and controlling the strength of hydrogen bonds is essential to control lignin solubility.<sup>62,69</sup>

Table 3. 3. Activity Coefficients and Lignin solubility of DES mixtures

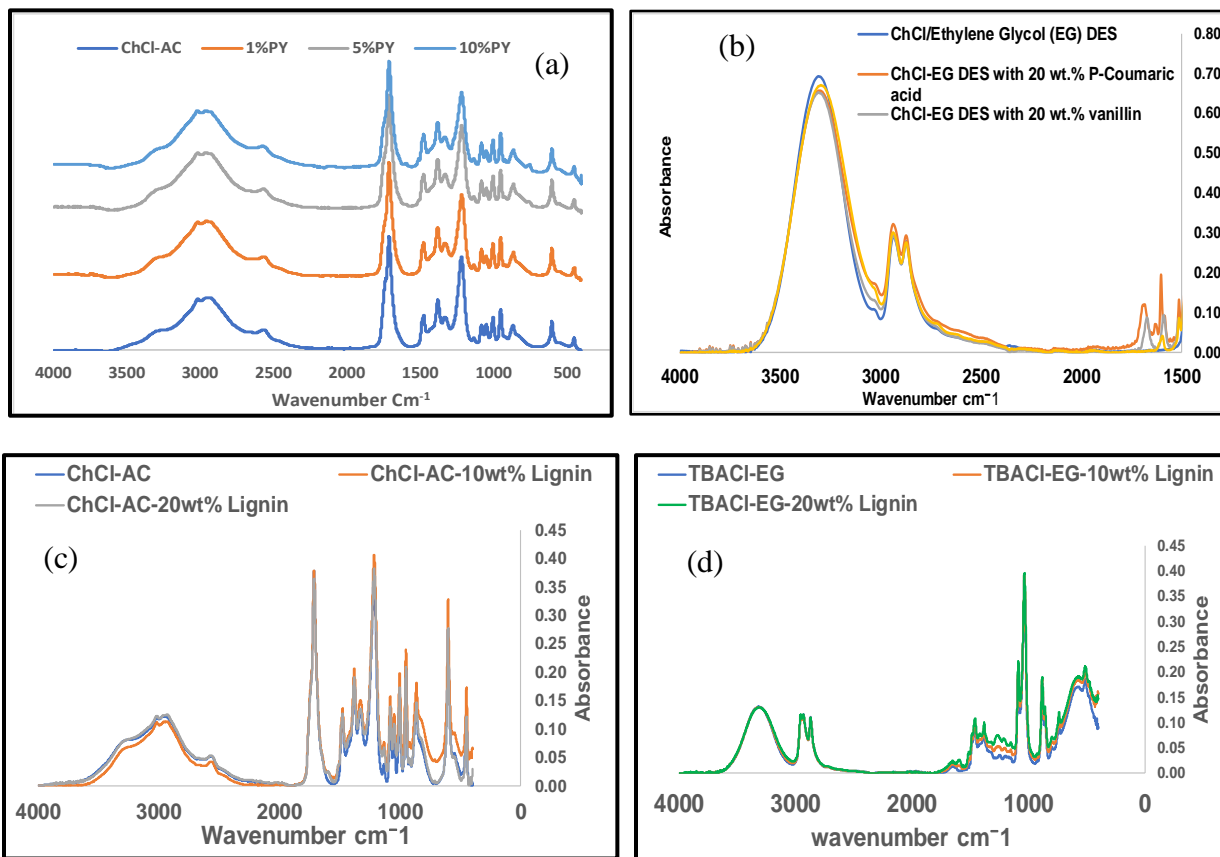
DES Type	Component		Activity Coefficient		Lignin Solubility at 31°C
	A	B	$\gamma_A$	$\gamma_B$	
ChCl/EG	ChCl	EG	0.992	0.343	1.26g/2.5g
ChCl/AC	ChCl	AC	0.699	0.290	1.52g/2.7g
ChCl/Lev	ChCl	Lev	0.696	0.371	0.86g/2.5g
sChCl/Py	ChCl	Py	0.676	0.091	0.002g/1g
TBACl/EG	TBACl	EG	0.252	0.270	0.99g/2.5g
TBACl/Hex	TBACl	Hex	0.188	0.466	0.005g/1g

### 3.6. General Heuristic III (Stability of DES toward Lignin monomers and Kraft lignin)

Understanding the stability of the developed DES was crucial for its effective use in the lignin fractionation process. In the context of extraction and separation, solvent stability is essential for enhancing recyclability, preserving product quality, and environmental impact among others.

The stability of the DES was tested with lignin monomers and kraft lignin to study the interaction between the DES and a third component. The FTIR spectra in Figure 3.5a-b revealed that DES is stable when 1%-20wt% lignin monomers were added to DES at 30°C for an hour under constant stirring. According to the FTIR spectra, the structure of DES did not show any significant

change under these conditions. However, ChCl/AC DES structure began to change when higher amount of lignin monomer (30wt % of pyrocatechol) was added. Similar stable behavior was noticed when 10%-20wt% kraft lignin (Figure 3.5c-d) was added to DES. This result further maximizes the potential use of DES for lignin fractionation and other applications.



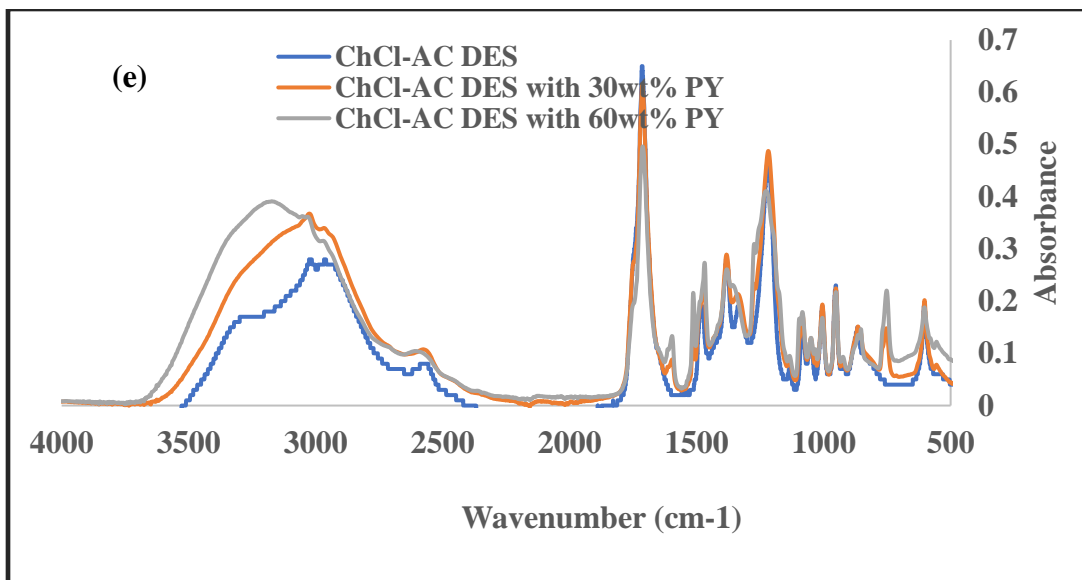


Figure 3. 5. shows FTIR spectra of DES plus different weight percentages of lignin monomer (pyrocatechol (PY), p-coumaric acid, vanillin) (a), (b) and (e) and kraft lignin (c) and (d).

### **3.7. Thermal Analysis**

#### **3.7.1 DSC**

Differential Scanning Calorimetry (DSC) is one of the thermoanalytical methods employed in lignin analyses where a sample's and a reference material's temperature is gradually raised at a constant rate. Next, the heat flow needed to increase the sample's and the reference material's temperatures at this steady rate is measured. This technique is useful in identifying phase transitions<sup>70</sup>. The glass transition temperature ( $T_g$ ) and specific heat capacity ( $C_p$ ) are important properties that affect lignin utilization as carbon fiber or thermoplastic. This study evaluated the  $T_g$  and specific heat capacity of the kraft lignin and the lignin fractions obtained after sequential fractionation using a single DES and sequential fractionation using multiple DES using DSC.

#### **Sequential Lignin Fractionation using Single DES (TBACl/EG, ChCl/Lev DES).**

For the single DES fractionated fractions, the DSC curves for TBACl/EG fractions and that of ChCl/Lev DES fractions are presented in Figure 3.6 through to Figure 3.8. Two antisolvents (DI water and acetone) were employed to precipitate lignin after fractionation.

For DI water precipitated fractions, the DSC curves depicted in Figures 3.6a and 3.7a below, reveal that,  $T_g$  values of all lignin fractions obtained after ChCl/Lev and TBACl/EG DES fractionation significantly reduced compared to the parent lignin (KL). KL presented two distinct  $T_g$  values of 71°C and 131°C. TBACl/EG DES lignin fractions exhibited the following  $T_g$  values, F1 73°C, F2 61°C, and F3 62°C. The undissolved fraction however did not show any distinct  $T_g$  value in this work suggesting that the undissolved fraction is more crystalline and thermally stable after fractionation. For the ChCl/Lev DES lignin fractions, F1 presented a  $T_g$  value of 65°C, F2



70°C, and F3 66 °C. Like the TBACl/EG undissolved fraction, the ChCl/Lev undissolved fraction presented no Tg value as shown in Figure 3.8 below.

Again, for acetone-precipitated fractions, acetone was found to precipitate ChCl/Lev DES and lignin out simultaneously. It could be that adding acetone to ChCl/Lev DES changes the DES's freezing point, resulting in the DES changing phases from liquid to solid as the lignin precipitates out. TBACl/EG on the other hand requires infinite dilution with acetone to precipitate lignin fractions out. The fractions exhibited the following Tg values, F1 63°C, F2 67°C, and F3 showing two distinct Tg values 58°C and 91°C, Figure 3.6b. The reduction in the Tg value of the lignin fractions compared to the parent lignin is an indication that the molecular weight of the fractions has been reduced because a higher Tg value can be related to higher molecular weight lignin.<sup>71</sup> This result is consistent with previous studies that explored the relationship between Tg and the molecular weight of a polymer.<sup>71,72</sup> According to these studies, an increase in Tg value means an increase in the polymers molecular weight which is explainable with the Flory-Fox equation.

$Tg = Tg_{\infty} - k/Mn$ , where  $Tg_{\infty}$  represents the highest Tg of the material at infinite molecular weight, k is a constant link with the free volume contained in the material, and Mn denotes the number-average molecular weight. These investigations have shown that a decrease in molecular weight causes the polymer free volume to rise, which in turn causes the Tg to decrease<sup>72</sup>. Another factor that has been noted to impact Tg value is the presence of hydroxyl groups. The presence of hydroxyl groups tends to increase hydrogen bonding which affects the glass transition temperature. This could be the reason TBACl/EG DI water precipitated fraction F1 showed a higher Tg value than F2 and F3. Lignin, however, does not always adhere to the Flory-Fox equation because of hydrogen bonding. However, the molecular weight impact on the Tg value is greater than the effect of the hydroxyl group.<sup>72</sup>

The reduction in the T<sub>g</sub> value in lignin fractions below 100°C enhances their efficient compatibility with synthetic thermoplastic polymers.

Using DSC curves, the study also examined the specific heat capacity of the various lignin fractions. The findings illustrated in Figures 3.7a, 3.7b and 3.8b show a rise in specific heat capacity in all lignin fractions precipitated with DI water. The rise was consistent with the trends seen in T<sub>g</sub> values. This implies that these fractions have improved thermal properties. Compared to KL, the fractions displayed a higher specific heat capacity, indicating enhanced thermal stability within the temperature range of 25°C to 200°C. Acetone was replaced with water as an antisolvent to precipitate lignin from DES. The DSC curves for the lignin precipitated with acetone displayed similar trends in T<sub>g</sub> value and specific heat capacity as the lignin precipitated with DI water. However, when precipitated with acetone, the F1 fraction showed a different behavior in terms of heat absorption.

Specifically, the heat capacity of F1 increased by about 150%, indicating that acetone precipitation yields a lignin fraction with higher thermal stability. A slight decrease in specific heat capacity was observed in the acetone-precipitated F3 fraction, while the F2 and the undissolved fractions remained unchanged. Comparing the T<sub>g</sub> values of DI water-precipitated fractions and acetone-precipitated fractions, the DI water-precipitated lignin fractions exhibited sharp T<sub>g</sub> temperature ranges, whereas the acetone-precipitated fractions exhibited broad T<sub>g</sub> temperature ranges. A broader T<sub>g</sub> range indicates that the fractions maintain their properties over a wider temperature range, further demonstrating that the acetone-precipitated lignin fractions are more thermally stable compared to the DI water-precipitated fractions.

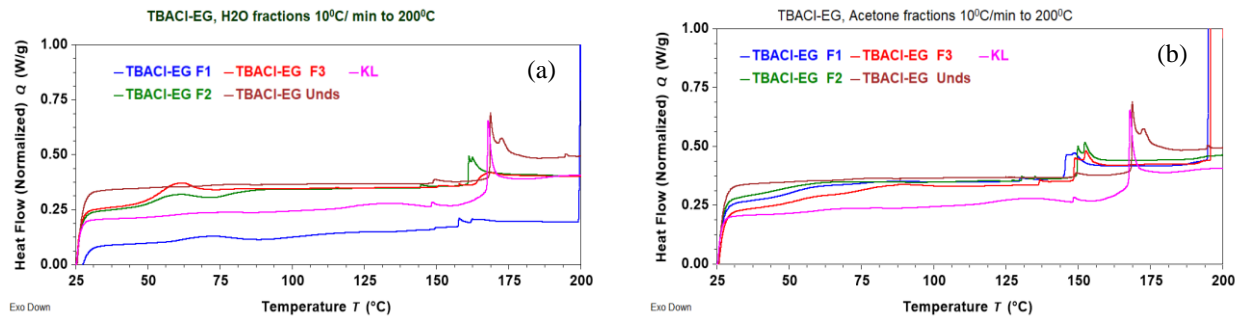


Figure 3. 6. Graphs showing the T<sub>g</sub> values of DI water precipitated lignin fractions (a) and acetone-precipitated lignin fractions (b) obtained with TBACl-EG DES fractionation.

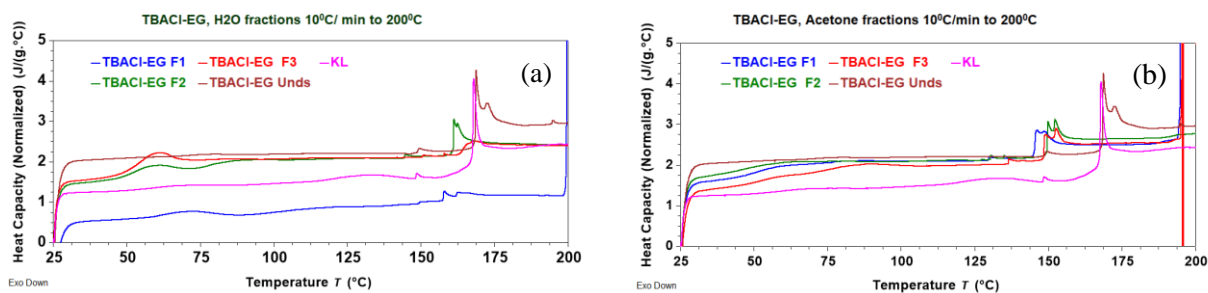


Figure 3. 7. Showing specific heat capacity of DI water and acetone precipitated lignin fractions obtained with TBACl-EG DES fractionation.

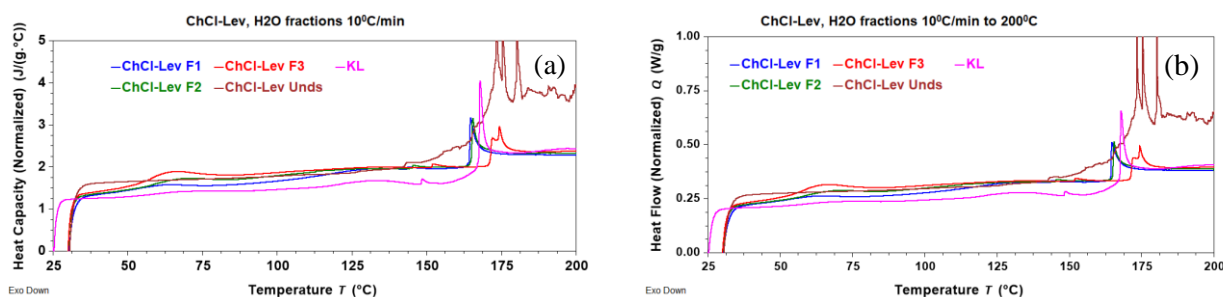


Figure 3. 8. Showing T<sub>g</sub> and specific heat capacity of DI water precipitated lignin fractions obtained with ChCl-Lev DES fractionation.

### Sequential Lignin Fractionation using Multiple DES.

Kraft lignin was sequentially fractionated using TBACl-EG DES followed by ChCl-Lev DES. TBACl-EG and ChCl-Lev DES soluble fractions were precipitated with DI water. The DSC curves showing the T<sub>g</sub>, and specific heat capacity values of the different fractions are presented in Figure 3.9. According to the DSC curves, the T<sub>g</sub> for the TBACl-EG soluble fraction was found to be 73°C and that of ChCl-Lev soluble fraction was 58°C. The ChCl-Lev soluble fraction exhibited a higher specific heat capacity OF 2.2 J/g°C at the glass transition temperature compared to the TBACl-EG soluble fraction which had a specific heat capacity of 0.8 J/g°C. This result clearly shows the ability of the DES to sequentially fractionate different fractions of KL.

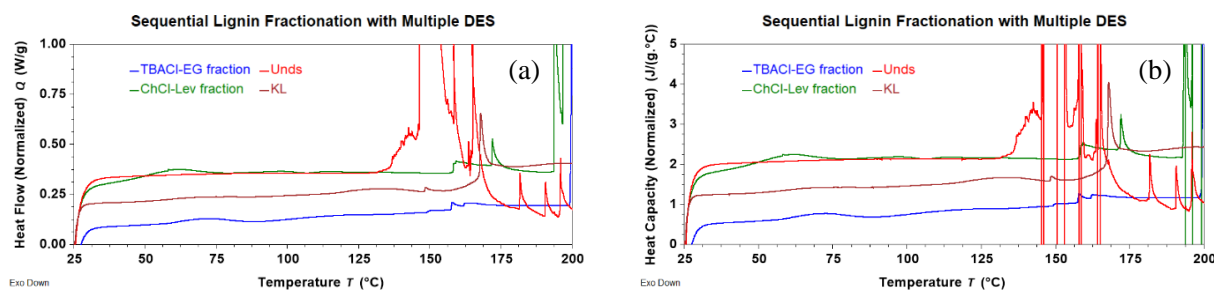


Figure 3. 9. DSC curve showing the T<sub>g</sub>, (a), and Specific heat capacity (b) of the KL, TBACl-EG, and ChCl-Lev soluble lignin fractions.

### 3.7.2 TGA

Thermogravimetric analysis (TGA) stands as a crucial thermoanalytical method in materials research, offering insights into a material's thermal stability, breakdown characteristics, and volatile content. In TGA, the mass of a sample is monitored as its temperature increases, either dynamically at a constant heating rate or isothermally over a predetermined duration.

This study employed TGA to investigate lignin fractions' degradation behavior and weight loss mechanism after DES fractionation. The TGA curves and derivative thermogravimetry (DTG) curves depicted in Figures 3.10b-a and 3.11a-b corroborate the findings of Ma et al. (2018) by illustrating lignin breakdown within specific temperature ranges. According to Ma et al. (2018),

lignin typically undergoes thermal degradation between 100°C and 800°C, with notable deterioration observed within specific temperature ranges. Following fractionation, all fractions (F1, F2, F3, and Undissolved) exhibited a greater weight loss in thermal degradation compared to the original lignin (KL) (Figure 3.9). The increase in weight loss can be attributed to the reduced molecular weight of the fractions after fractionation. Additionally, previous research suggests that functional groups, such as hydroxyl and methoxy groups, influence lignin degradation behavior. Generally, lignin with higher molecular weight tends to yield decreased residue, whereas the amount of carbonized residue increases as the concentration of functional groups decreases<sup>73</sup>. The fractions obtained through ChCl-Lev DES exhibited percentage weight losses within the temperature range of 100-800°C as follows: 71% for F1, 55.9% for F2, 59.49% for F3, and 74.5% for the undissolved fraction. In contrast, for TBACl-EG DES, the weight loss percentages were observed as follows: the undissolved fraction experienced the highest weight loss at approximately 84%, followed by F1 at 77%, F3 at 70%, and F2 at 65%. Comparatively, kraft lignin exhibited a weight loss of approximately 52% at 800°C, retaining more weight due to its higher molecular weight compared to the fractions.

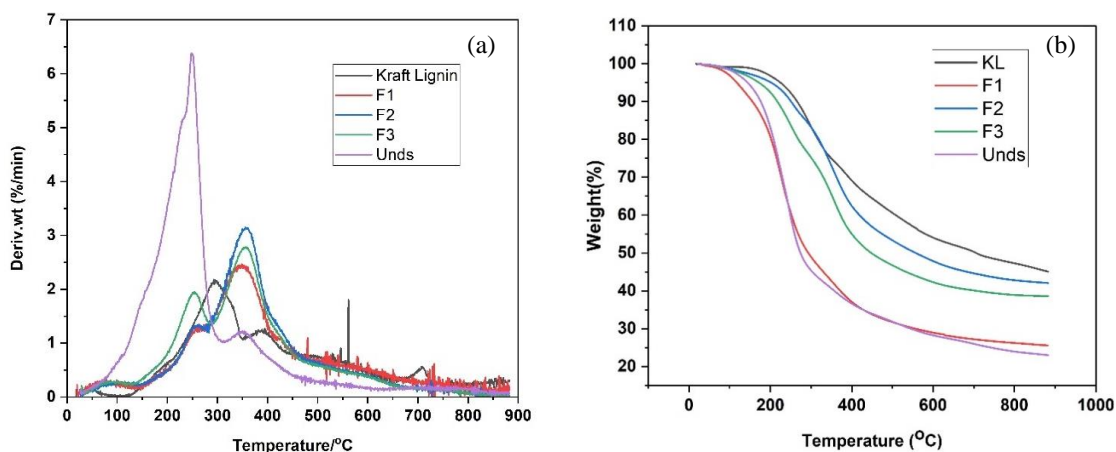


Figure 3. 10. DTGA (a) and TGA (a) of kraft lignin and ChCl-Lev lignin fraction.

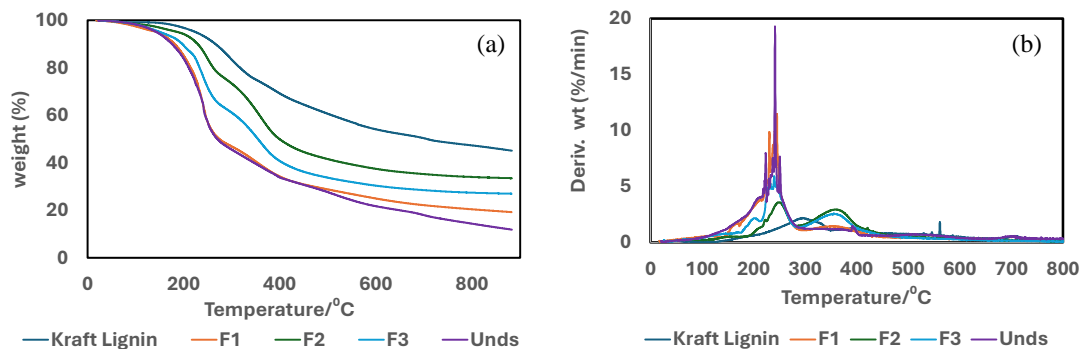


Figure 3. 11. TGA (a) and DTGA (b) of Kraft lignin and TBACl-EG lignin fractions.

### 3.8. Functional Group Analysis Lignin Fractions.

#### 3.8.1 Single DES Fractionation Lignin Fractions

<sup>1</sup>H NMR spectra were used to characterize the structural characteristics of the fractionated lignin samples. Figure 3.12 displays the corresponding integral assignments of the signals. The signals corresponding to aromatic protons fall between 6.0 and 8.0 ppm. In particular, the aromatic protons in p-hydroxyphenyl structures were linked to the signal at 7.5–7.3 ppm, whereas the aromatic protons in guaiacyl structures were linked to the signal at 7.3–6.8 ppm.<sup>74</sup> No syringyl protons were identified in the lignin sample. This is because the kraft lignin fraction is a softwood characterized by guaiacyl units mainly. The aromatic ring's side chains include aliphatic protons, which are responsible for the peaks that emerge between 4.2 and 6.3 ppm. The strong changes between 4.0 and 3.5 ppm are linked to methoxyl protons found in all lignin fractions; water protons are linked to 3.3 ppm, whereas DMSO protons are linked to 2.5 ppm. All lignin fractions contain aliphatic hydrogens, as indicated by the spectral bands between 1 and 1.7.<sup>75</sup> Due to variations in phenolic hydroxyl concentration, methoxyl hydroxyl group content, and molecular weight, the signal intensities differed amongst the lignin fractions.

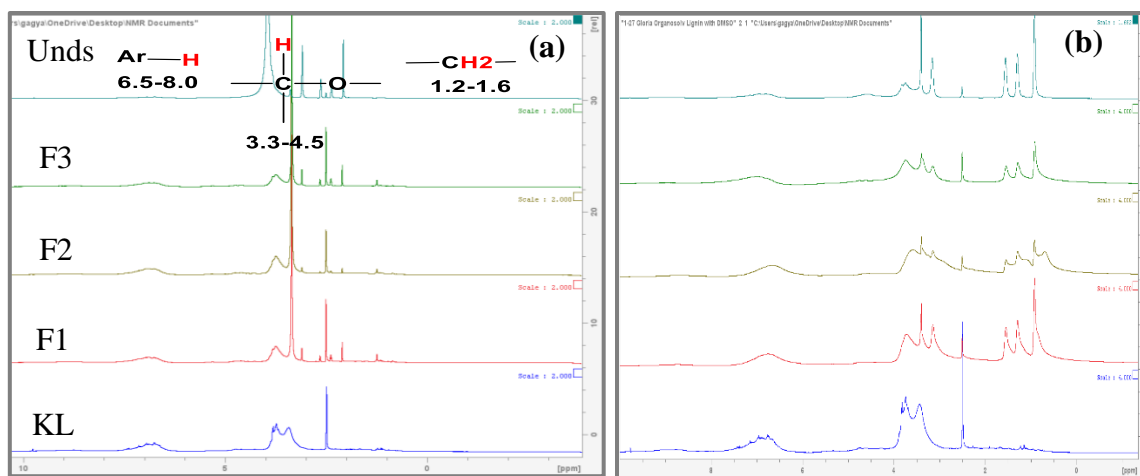


Figure 3. 12. H-NMR of Kraft lignin and ChCl-Lev lignin fractions (a), kraft lignin and TBACl-EG lignin fractions (b).

### 3.8.2 Multiple DES Fractionation Lignin Fractions

Similar to the single DES fractionation lignin fractions, the fractions obtained after multiple DES fractionations presented different functional groups. This result indicates that multiple DES fractionations represent an efficient and convenient way of obtaining lignin fractions with different functional groups. The ChCl-Lev soluble fraction had aromatic methyl groups which correspond to the peak's signals between 2.4–2.7 ppm. The undissolved fractions also exhibited the same peaks, while TBACl-EG had no aromatic methyl hydrogen. In addition, all the fractions contained aliphatic hydrogen groups, (alkyl hydrogen) however, the intensity of the alkyl hydrogens, linked to the peaks between 1.4–1.8 ppm decreased from TBACl-EG soluble fraction to the undissolved fraction. Again, the signals corresponding to aromatic protons fall between 6.0 and 8.0 ppm. In particular, the aromatic protons in p-hydroxyphenyl structures were linked to the signal at 7.5–7.3 ppm, whereas the aromatic protons in guaiacyl structures were linked to the signal at 7.3–6.8 ppm.<sup>74</sup> The strong changes between 4.0 and 3.5 ppm are linked to methoxyl protons found in all

lignin fractions; water protons are linked to 3.3 ppm, whereas DMSO protons are linked to 2.5 ppm.

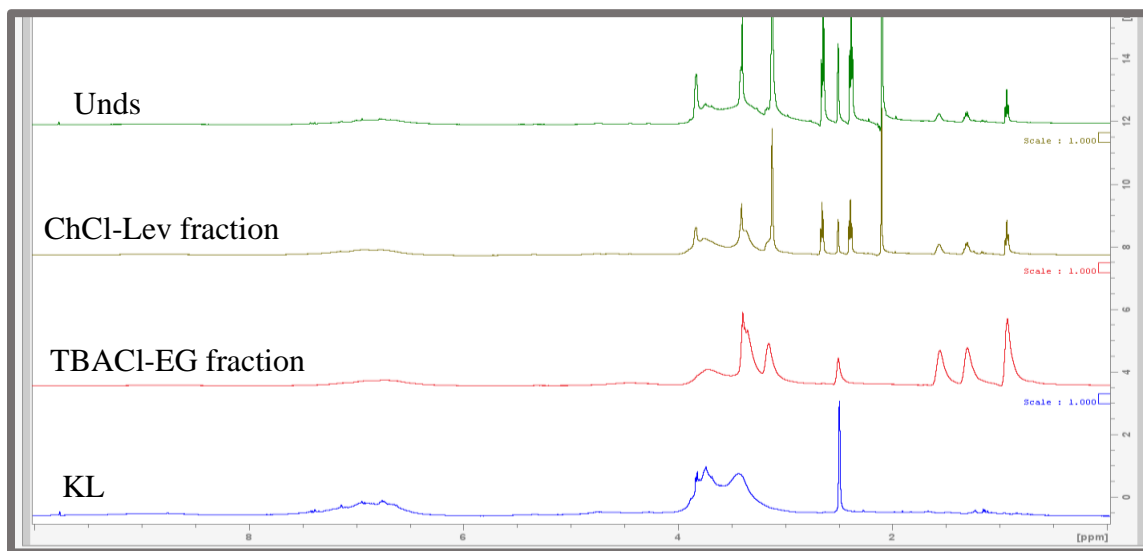


Figure 3. 13. H-NMR spectra of KL, TBACl-EG, and ChCl-Lev soluble lignin fractions.

### 3.9. Elemental Analysis of lignin fractions

The lignin fractions obtained through ChCl-Lev DES fractionation exhibited a reduction in carbon percentage and an increased in hydrogen content relative to the parent lignin (kraft lignin). Table 3.4 which represent the elemental analysis of ChCl-Lev DES lignin fractions and Table 3.5, elemental analysis result for TBACl-EG DES lignin fractions indicate that kraft lignin contained approximately 63.46% carbon and 5.51% hydrogen. F1 displayed the highest carbon content and the lowest hydrogen content at 62.64% and 5.61%, respectively. F2 exhibited a slightly lower carbon content and a slightly higher hydrogen content compared to F1 (62.14% carbon, 5.75% hydrogen). F3 and the undissolved fractions demonstrated significant variations in their carbon and hydrogen content, with carbon and hydrogen percentages of 59.49%, 5.84%, and 54.26%, 5.82%, respectively.



The nitrogen content did not exhibit a discernible trend. In the case of TBACl-EG DES fractions, F2 displayed the lowest carbon content, followed by undissolved, F3, and F1, with percentages of 56.41%, 59.75%, 62.95%, and 64.89%, respectively. The hydrogen composition increased across the fractions except for F2, which showed a decrease. This variance might be attributed to the structural complexity of lignin and its inherent inconsistencies. A similar trend was observed in nitrogen composition. The undissolved fraction exhibited the highest hydrogen content at 8.52%, followed by the F3 fraction at 7.33%, F2 at 6.62%, and F1 at 6.87%, respectively.

Table 3. 4.CHN of Kraft lignin and ChCl-Lev DES lignin fractions

Sample	%C	%H	%N
Kraft lignin	63.46 ± 1.28	5.51 ± 0.31	0.51 ± 3.29
F1	62.64 ± 0.08	5.61 ± 0.35	0.392 ± 5.33
F2	62.14 ± 0.69	5.75 ± 2.914	0.54 ± 35.92
F3	59.49 ± 1.15	5.84 ± 0.57	0.81 ± 4.188
Undissolved	54.26 ± 0.48	6.82 ± 2.23	2.17 ± 42.32

Table 3. 5.CHN of Kraft lignin and TBACl-EG DES lignin fractions

Sample	%C	%H	%N
Kraft lignin	63.46 ± 1.28	5.51 ± 0.31	0.51 ± 3.29
F1	64.89 ± 1.42	6.87 ± 5.27	1.05 ± 9.3
F2	56.41 ± 6.33	6.62 ± 7.63	0.96 ± 15.02
F3	62.95 ± 1.76	7.33 ± 2.38	1.26 ± 7.05
Undissolved	59.75 ± 1.74	8.52 ± 2.73	1.72 ± 5.14

## CHAPTER 4

### SUMMARY AND RECOMMENDATIONS

#### 4.1. Conclusion

Seven deep eutectic solvents were developed (ChCl/Lev, ChCl/EG, ChCl/AC, ChCl/Py, TBACl/EG, TBACl/Hex, Oct/Hex) using the simple heating and mixing method. Freezing point measurement, FTIR, and Activity coefficient calculation characterized all DESs. All the DESs presented a temperature depression below that of their pure constituents at their eutectic composition. Also at the eutectic point, the mixtures presented negative deviations to ideality suggesting a stronger interaction existing between the mixtures. These interactions were also evident in the FTIR spectra in which the OH stretches of DES showed a broadening of the peak because of OH interaction between the HBDs and the HBAs. A shift in peak position is also an indication that an interaction has occurred in the system.

These results indicated a successful preparation of DES. Three general heuristics were developed based on DES characterization using activity coefficient measurement, DES stability test with lignin monomers, and kraft lignin, and the ability of DES to solubilize lignin. First, at the eutectic point, it was observed that the activity coefficient of the components involved in the DES formation was less than 1. Thus, the HDB and the HBA of each DES, at the eutectic composition deviate negatively from thermodynamic ideality. Secondly, lignin solubility in DES was found to be minimal when either of the components deviated below about ( $\gamma < 0.2$ ) suggesting that there is an extent to which these components should deviate negatively.

The last general heuristic was that all synthesized DES exhibited stability toward about 20 weight percent lignin monomers (vanillin, pyrocatechol, p-coumaric acid) as well as the kraft lignin. Two of the developed DESs (ChCl/Lev, TBACl/EG) were employed for the fractionation

of kraft lignin. Four fractions, designated as F1 through undissolved, were isolated from the parent lignin sequentially with a single DES (ChCl/Lev or TBACl/EG). In addition, kraft lignin was sequentially fractionated using TBACl/EG followed by ChCl/Lev DES. Analysis via H-NMR revealed a decrease in methoxyl and phenolic hydroxyl group content, corresponding to a reduction in peak intensity because of the decrease in molecular weight of fractions for single DES fractionation lignin fractions. In the case of lignin fractions obtained after sequential fractionation with TBACl/EG and ChCl/Lev, H-NMR revealed that the ChCl/Lev soluble fraction exhibited aromatic methyl groups, identified by peaks between 2.4–2.7 ppm. These same peaks were also present in the undissolved fractions, whereas the TBACl/EG fraction lacked aromatic methyl hydrogen.

Additionally, all fractions contained aliphatic hydrogen groups (alkyl hydrogen), but the intensity of the alkyl hydrogen peaks between 1.4–1.8 ppm diminished from the TBACl/EG soluble fraction to the undissolved fraction. Thermal analyses conducted through TGA/DTGA, and DSC studies indicated that all lignin fractions after fractionation exhibited improved thermal properties as indicated by higher specific heat capacity within the temperature range of 25 to 200°C compared to the parent KL as evidenced by DSC results. These results suggest the molecular weight of the lignin fractions has been reduced after fraction. Again, lower T<sub>g</sub> value below 100°C observed in lignin fractions makes the fractions more compatible for blending with synthetic thermoplastic polymer matrices. Elemental analysis of all lignin fractions showed a reduction in carbon content and an increase in hydrogen content. In conclusion, these findings suggest that using both a single DES and multiple DES sequentially for lignin solvent fractionation is effective in obtaining more homogeneous lignin fractions. Each lignin fraction obtained through this method

demonstrates distinct physical and chemical characteristics, highlighting the potential of this approach for lignin processing.

#### **4.2. Recommendations and future work**

DESs hold enormous potential as a green solvent for lignin fractionation and valorization. To enhance their efficiency, it is essential to comprehend how DES interacts with lignin during fractionation, dissolution, and valorization processes. It is also crucial to clarify the molecular mechanisms underlying the breakdown and separation of lignin during fractionation. Further study should aim at understanding the inherent processes and structural changes lignin undergoes during fractionation. Additionally, a correlation between solvent polarity and the structure of the obtained lignin fractions should be explored further. Evaluating the yield of each fraction can lead to more effective lignin fractionation.

Moreover, examining the impact of adding water to DESs on their molecular structure and interaction with lignin could provide another pathway to designing DES for lignin fractionation. Given the vast variety of DES forming components, efficient screening techniques are essential for selecting the most effective solvents for lignin fractionation. Although research on acidic DESs is now dominating, type V DESs and other types are equally promising and can be explored for their effectiveness for lignin fractionation. DES selection can be efficiently guided by utilizing the general heuristics developed in this study as well as computational techniques like machine learning, which can manage massive datasets. It is essential to develop effective recycling solutions for DESs.

This study has revealed that DESs undergo compositional changes, degradation, and unanticipated interactions with contaminants formed during lignin fractionation, precipitation with antisolvents and from lignin during recyclable usage, which affects their characteristics. Therefore,

more investigation is required to comprehend the basic characteristics and chemical structures of DESs to minimize unforeseen interactions and investigate cutting-edge impurity removal technologies.

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## APPENDIX A

### **Biorefineries**

A biorefinery, analogous to a conventional petroleum refinery, is a facility that integrates various biomass conversion processes and technologies to produce a variety of bio-based products, fuels, and chemicals, as illustrated in Figure A.1. The main feedstocks in a biorefinery include a variety of biomass resources such as forestry residues (wood chips, and sawdust), crops and residues (like corn, corn stover, rice, rice straw, wheat straw, sugarcane bagasse) dedicated energy crops (like switchgrass), algae, and organic waste, as its feedstock instead of fossil fuels. Not only are biorefinery processes environmentally friendly, but also sustainable as they offer an alternative to traditional fossil-based products mitigating climate change.<sup>76</sup> Ultimately, the goal and purpose of the integrated biorefinery is to serve as an alternative petroleum refinery in producing fuel, materials, and chemicals to meet the world's demand by creating a more environmentally friendly and sustainable pathway for developing these products. Its main goal is to create specialty chemicals with high value, low market volume, and more commodity chemicals using different production methods. These methods are made to get the most value out of the products and make less waste by turning common products into energy. The valuable products can make more money, and the common products can help meet the world's energy needs. The electricity made by a biorefinery can also cut down on overall costs. In a biorefinery, biomass like plants and organic waste is used to make useful things like fuel, power, and chemicals through processes like biology and chemistry. In the past, biorefineries mainly used bioconversion (like aerobic and anaerobic digestion) and chemical conversion. There are currently two new and promising biorefinery types: the sugar platform and the thermo-chemical platform (syngas platform). In sugar biorefineries, biomass is broken down into different sugars for making fuels and chemicals through fermentation

or other processes. In thermo-chemical biorefineries, biomass is turned into hydrogen, carbon monoxide, or pyrolysis oil, which can be used directly as fuel.

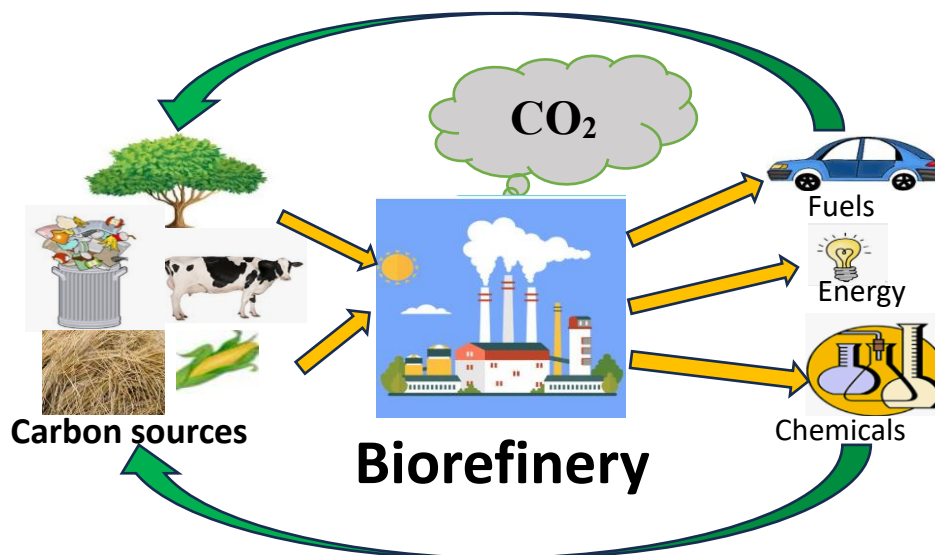


Figure A. 1. The Concept of Biorefinery.

### Lignocellulosic Biomass

The most abundant renewable material on earth is Lignocellulosic biomass, also known as lignocellulose. It is produced from atmospheric CO<sub>2</sub> and water with sunlight energy through photosynthesis. Lignocellulose is an important part of the woody plant's cell wall, comprising a complex mixture of polysaccharides, phenolic polymers, and proteins. Its structure is complicated, with cellulose, a carbohydrate polymer, complexly interrelated to hemicellulose, another carbohydrate polymer, and lignin, an aromatic polymer as illustrated in Figure A.2. Lignocellulosic biomass (LCB) is typically classified into three categories: virgin biomass, waste biomass, and energy crops.<sup>77</sup> Virgin biomass includes trees, bushes, and certain grasses, while agricultural residues like rice straw, stover, and bagasse are classified as waste biomass. Energy crops are specifically grown for their high biomass productivity, primarily used to produce second-generation biofuels. LCB has a rich history as an energy source. For many centuries, wood served

as the primary material for burning fires. However, during the Industrial Revolution, the escalating energy demand led to a transition from wood to fossil fuels. As concerns grew over pollution and the depletion of fossil fuel reserves in the mid-20th century, there has been a resurgence in the use of biomass for energy production. This shift has been driven by the need for more sustainable and renewable energy sources.<sup>77</sup> Lignocellulose is composed of primarily Cellulose, hemicellulose, lignin, extractives, and ash. These are grouped into two: the carbohydrate (cellulose and hemicellulose) and the non-carbohydrate (lignin, extractives, and ash). The carbohydrates are made up of about 70% lignocellulose dry weight, 25% lignin, and about 5% extractive and ash content.<sup>77</sup> Cellulose is the most abundant organic compound on earth composed of carbon, hydrogen, and oxygen. It is a macromolecular compound with the chemical formula  $(C_6H_{10}O_5)_n$ . It is found in the cell walls of plants, algae, and oomycetes. Its purpose is to give the plant structural support. It is a homopolysaccharide, made up of D-glucose molecules that are linked through  $\beta$ -(1,4) glycosidic bonds. Cellulose content in wood is about 40%-50%.<sup>77</sup>

Hemicellulose is like cellulose but differs in structure and characteristics. It is a heteropolysaccharide found in the cell walls of plants where it provides strength. Unlike cellulose, hemicelluloses are composed of different sugars such as pentose (xylose, arabinose), hexoses (mannose, galactose, glucose, and rhamnose), acetylated sugars, etc. Their structure is more branched with about 500 to 3000 sugar units. Hemicelluloses are complex polymers that cross-link with either cellulose or lignin within the plant cell wall. They are more soluble in water than cellulose and they are mostly found in cereal grains like oats and wheat, grasses, wood, and some fruits and vegetables.<sup>77</sup> Lignin is the second most abundant natural polymer on earth, surpassed only by cellulose. It is a complex aromatic macromolecule that serves as an important constituent of plant cell walls, providing structural support and rigidity. Lignin is found in all vascular plants

and accounts for approximately 20-35% of the dried biomass of wood. Lignin is composed of three main methoxylated phenyl propane units, also called phenyl propane units or monolignols, as shown in Figure A. 4. In the plant cell wall, lignin is covalently bonded to the carbohydrates through phenyl glycosides, benzyl esters, and benzyl ethers bonds. The average empirical formula for lignin,  $C_9H_{10}O_2(OCH_3)_n$ , is a representation of its complex chemical structure and is derived from the phenylpropanoid unit.<sup>78</sup>

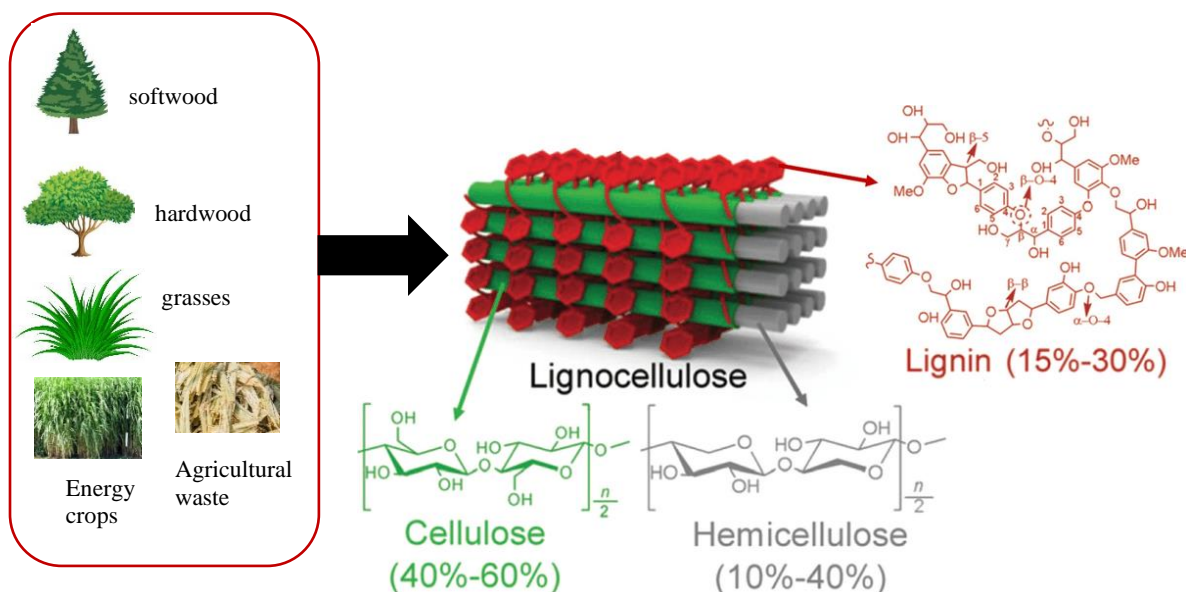


Figure A. 2. Major components of lignocellulose material. Modified from Akhundi et al. (2022).<sup>79</sup>

#### 4.2.1.1 Lignin biosynthesis and structure

Lignin is a complex aromatic macromolecule that is formed through the radical coupling polymerization of three main monolignols, the p-Coumaric alcohol, Coniferyl alcohol, and the Sinapyl alcohol as depicted in Figure 4.4.<sup>80</sup> It has the most complex structure among the three main components of lignocellulose biomass. It is neither a polysaccharide nor a protein, but rather a phenolic polymer. The precursors of lignin are produced via the shikimic acid pathway described in Figure A.3, which is also involved in the metabolism of carbohydrates and aromatic amino acids, along with many other aromatic elements of plants. Phenylalanine, an essential step in the

production of lignin, is produced via this pathway.<sup>78,81</sup> Three hydroxycinnamyl alcohols, or monolignols, are produced during the reaction: p-coumaryl, coniferyl, and sinapyl. These subsequently yield the lignin subunits 4-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), in that order. The positioning of methoxy groups and the extent of substitution on the ring are the main structural differences between them. These three monolignols undergo enzymatic oxidative polymerization to form the structure of lignin. Enzymatic oxidation of the monolignol phenolic hydroxyl groups is the first step in the polymerization process. Radical sites are created by enzymes like laccases or peroxidases. An electron is transferred at the start of this enzymatic dehydrogenation process, producing reactive monolignol species that include free radicals that can then mix. The aromaticity of the benzene ring is then restored by nucleophilic assault on the benzylic carbon of the quinone methide intermediate by alcohols, water, or phenolic hydroxyl groups. Subsequent polymerization is then applied to the resultant dilignols.<sup>82</sup>

These three monolignols or the phenyl-propanol units are chemically linked primarily by ether linkages (e.g.,  $\alpha$ -O-4, 5-O-4, and  $\beta$ -O-4) shown in Figure 1.3.<sup>83</sup> In addition to the ether linkages, a variety of intermolecular linkages exist also between lignin molecules which further complexes the structure of lignin. These include the condensed linkages (e.g., 5-5,  $\beta$ - $\beta$ ,  $\beta$ -5, and  $\beta$ -1 linkages). About 40-60% of the intermolecular bonds are ether bonds of which the  $\beta$ -O-4 is the predominant.<sup>84</sup> The molecular weight, composition, and amount of lignin monolignol differ from plant to plant.<sup>85</sup> The composition of lignin varies depending on its origins, with softwood lignin predominantly derived from G-type monolignol, hardwood lignin mainly derived from G- and S-type monolignols with traces of H-type monolignol<sup>86</sup> and the lignin obtained from grasses containing a substantial amount of each monolignol. This variation in monolignol composition contributes to the diversity of lignin structures found in different plant species. In addition to the

main monolignols, lignin also contains small amounts of units from incomplete monolignol biosynthesis and various other phenylpropanoid units.<sup>87</sup> These additional phenylpropanoid units, such as hydroxycinnamyl aldehydes, acetates, p-coumarates, p-hydroxybenzoate, and tyramine ferulates are integrated into the lignin polymer. The polymerization of monolignols is random, resulting in a highly plastic lignin composition that can be manipulated.<sup>87</sup> Additionally, lignin is an aromatic biopolymer rich in multifunctional groups such as -OH, -OCH<sub>3</sub>, -COOH, and -C=O.<sup>88</sup>

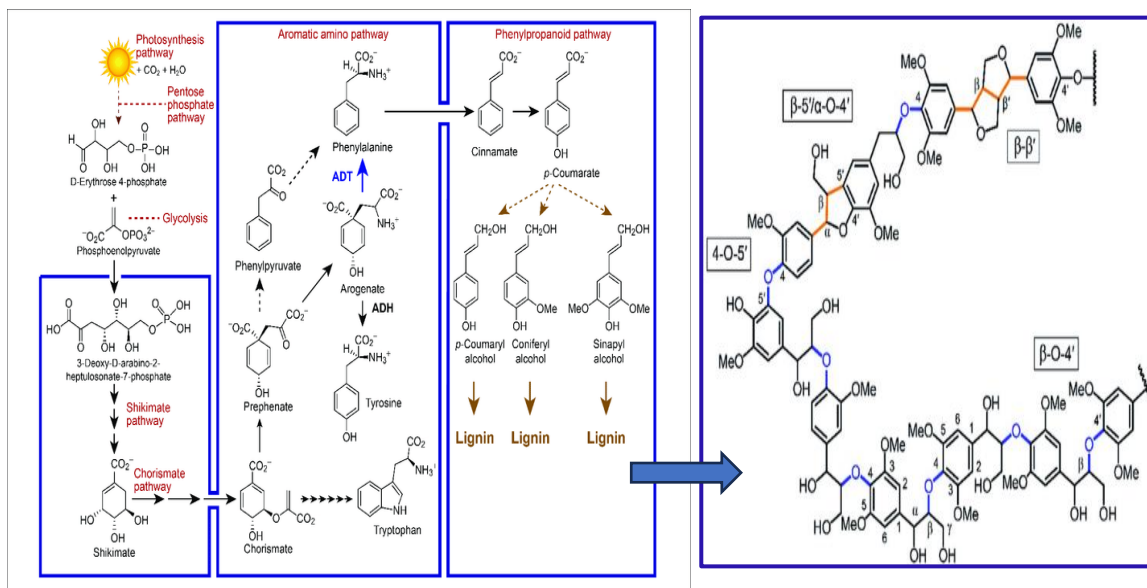


Figure A. 3. Lignin biosynthesis pathway and a model showing the interunit ether connections in blue and the C-C linkages in orange, illustrating the structure of hardwood lignin. The phenylpropanoid structures in between these links correspond to lignin units. Reproduce from Amiri et al. (2019).<sup>89</sup>

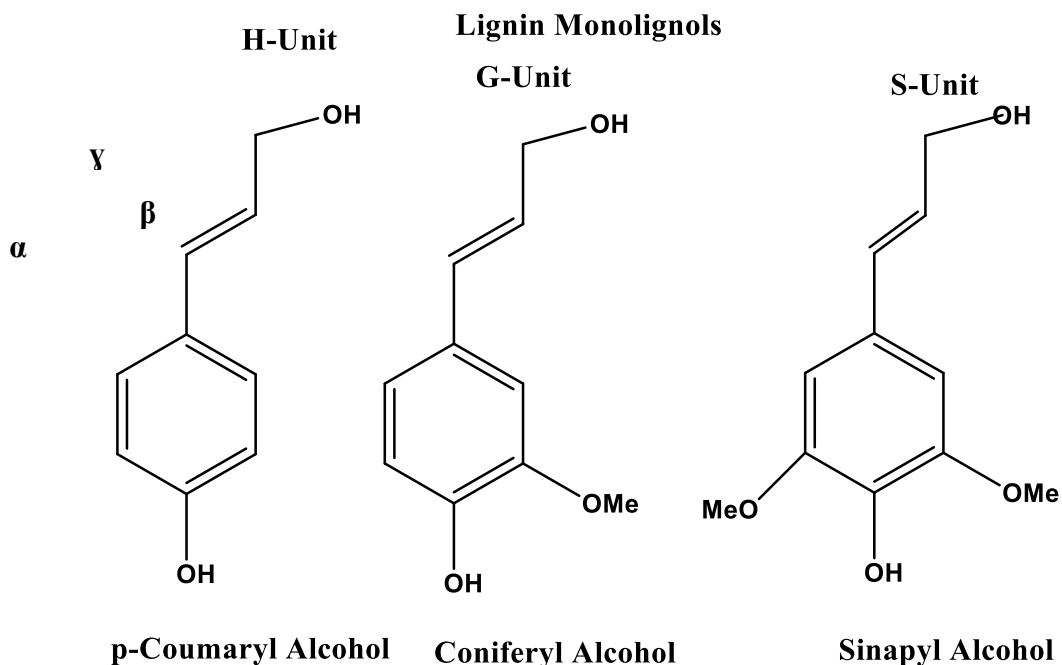


Figure A. 4. Structures of the three main monolignols of lignin.

#### 4.2.1.2 Types of Lignin/Lignin extraction processes

In industry, lignin extraction is categorized into two processes: the sulfur Process (lignosulfonates, kraft lignin) and the sulfur-free process (alkaline, organosolv lignin). The extracted lignin, often termed technical lignin, is significantly influenced by the extraction method.

Typically, technical lignin is called after the process of extraction, examples include soda lignin, kraft lignin, organosolv lignin, and lignosulfonate. Lignin separation methods fall into two categories: (1) delignification, which primarily uses chemical means to extract lignin from biomass, leaving cellulose and hemicellulose as delignified pulp. Lignin obtained from this isolation method is either isolated as a solid lignin precipitate (LP) or as a depolymerized lignin (DL) as determined by the method used in the isolation. (2) The second separation method focuses on hemicellulose and cellulose conversion. Here, lignin is isolated as an insoluble lignin residue (LR) or as a lignin precipitate (LP).<sup>90</sup> While the insoluble form of lignin maintains the original

morphological structure of the raw material fibers, the soluble form has an amorphous structure.<sup>80</sup> The main commercial source of lignin is the pulp industry, and the biorefinery idea is beginning to acknowledge lignin as a valuable by-product.<sup>91,92</sup> According to<sup>93</sup> lignin extraction methods use organic solvents or acidic and alkaline media to gradually break the lignin into low molecular weight pieces and alter its physical-chemical characteristics. Significant degradation and structural change of native lignin is caused by the different extraction procedures because lignin can partially degrade during the extraction process, the separation techniques also affect the molecular weight and polydispersity of lignin. Furthermore, factors like mechanical action, enzymes, or chemical reagents might affect the crosslinked structure of lignin and the outcome of the separation leading to decreased amounts of aliphatic OH groups,  $\beta$ -O-4, and  $\beta$ - $\beta$  linkages. On the other hand, the development and/or accumulation of condensed lignin structures is generally associated with a rise in phenolic hydroxyl groups, carboxylic acids, and carbonyl groups, as well as an elevated degree of condensation.<sup>78</sup> As a result, the extraction technique, and the plant species (softwood, hardwood, or grasses) usually affect the structure of technical lignin.<sup>78</sup> Technical lignin therefore requires further fractionation before its utilization in high-value products as illustrated in Figure 4.7. Some common and widely used types of lignin are described below:

#### **Kraft lignin /Alkali Lignin.**

These types of lignin are produced from biomass through the kraft pulping process. In this process, high pressure and temperature are applied in the presence of NaOH solution. Under these conditions, the linkages between the lignin and the carbohydrates are broken, creating lignin fragments that dissolve in the NaOH solution to form a black liquor as depicted in Figure A.5. The lignin is then extracted from the black liquor using either Na<sub>2</sub>CO<sub>3</sub> or Na<sub>2</sub>SO<sub>4</sub> to form Alkali or Kraft lignin respectively.<sup>35</sup> The kraft pulping process is currently the most used in the pulping



industries because the process is economically feasible as most of the chemicals used in the process are recoverable. In addition, the kraft process works for all plant species. As a result, most of the technical lignin is produced using the kraft process resulting in a 50 million tons annual kraft lignin generation. In a traditional kraft pulping process, wood chips are processed in a sizable pressure tank called a digester. There, the wood chips react with an aqueous solution of sodium hydroxide and sodium sulfide, or "white liquor." The mixture is brought to a temperature of around 170°C and left there for about two hours.<sup>82,94</sup> The lignin hydroxide and hydrosulfide ions react causing the polymer to break down into smaller, water- and alkali-soluble pieces. The lignin macromolecule breaks down by cleaving the bonds that hold the phenylpropane units together, which releases phenolic hydroxyl groups.<sup>82</sup> These hydroxyl groups improve the lignin's and its fragments' hydrophilicity, which makes them more soluble in the cooking liquid. The more durable carbon-carbon bonds, however, are typically held throughout the pulping procedure. After a typical softwood kraft boil, the residual or leftover lignin which makes up around 4-5 percent of the total weight is usually eliminated using bleaching methods.<sup>82</sup> Lignin reactions in kraft pulping fall into two main categories: degradation and condensation reactions. Degradation reactions are advantageous because they release fragments of lignin and make it easier for them to dissolve. On the other hand, condensation reactions produce alkali-stable links, which are undesirable because they increase the recalcitrance of lignin.<sup>82</sup>

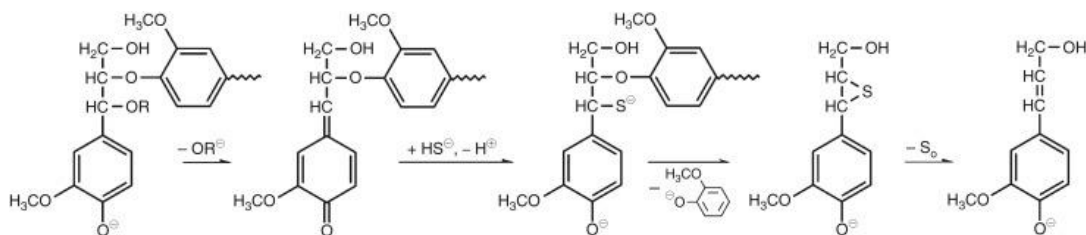


Figure A. 5. Kraft lignin process. Adapted from Fernández-Rodríguez (2018).<sup>19</sup>

## **Organosolv Lignin**

Organosolv lignin is the type of lignin isolated from lignocellulose biomass using the organosolv process. The organosolv process is one of the acidic lignification methods that focus on extracting lignin from the biomass matrix using organic solvents at temperatures ranging from 140 to 220 °C using HCl as a catalyst in the presence of water as illustrated in Figure A.6.<sup>80</sup> Since its development in the 1970s, it has been employed widely by the pulp and bioethanol industries for biomass fractionation.<sup>95</sup> The organosolv lignin offers several advantages over other lignin extraction methods. This includes extraction of lignin with high purity, and high chemical reactivity, and the extraction of sulfur-free lignin, depending on the solvent used, and has a low molecular weight. In addition to these advantages, organosolv lignin is also easily recoverable, making it a cost-effective and sustainable option for lignin extraction.<sup>96</sup> The solvents used in the organosolv process selectively remove lignin from lignocellulosic material, vis-a-vis wood chips or agricultural residues. According to Goncalves et al. (2021) the process works by cleaving the carbohydrate-lignin bonds preferably, leading to the extraction of high-molecular-weight lignin without significant chemical modifications.<sup>96</sup> The structure of the organosolv lignin is affected by the specific organic solvent used in the extraction process. Also, the choice of solvent can influence the properties of the extracted lignin and determine its suitability for specific applications. Some of the widely used solvents as reported by Solihat et al. (2021) include alcohols (such as ethanol and methanol), organic acids (such as acetic acid), ketones (such as acetone), and esters (such as ethyl acetate).<sup>97</sup> The solvent concentration in water typically ranges from 40% to 80%. Numerous organosolv methods have been reported, resulting in lignin commonly referred to as organosolv lignin. Four main organosolv pulping processes include the Organocell process, Alcell process, alkaline sulfite-antraquinone-methanol (ASAM) process, and acetosolv process.<sup>80,98</sup> Studies have

shown that the organosolv process with an ethanol-water mixed solvent produces a higher pulp yield compared to the kraft process, with the tensile and tear strength of softwood organosolv pulps falling between the corresponding values of kraft pulp and sulfite pulp.

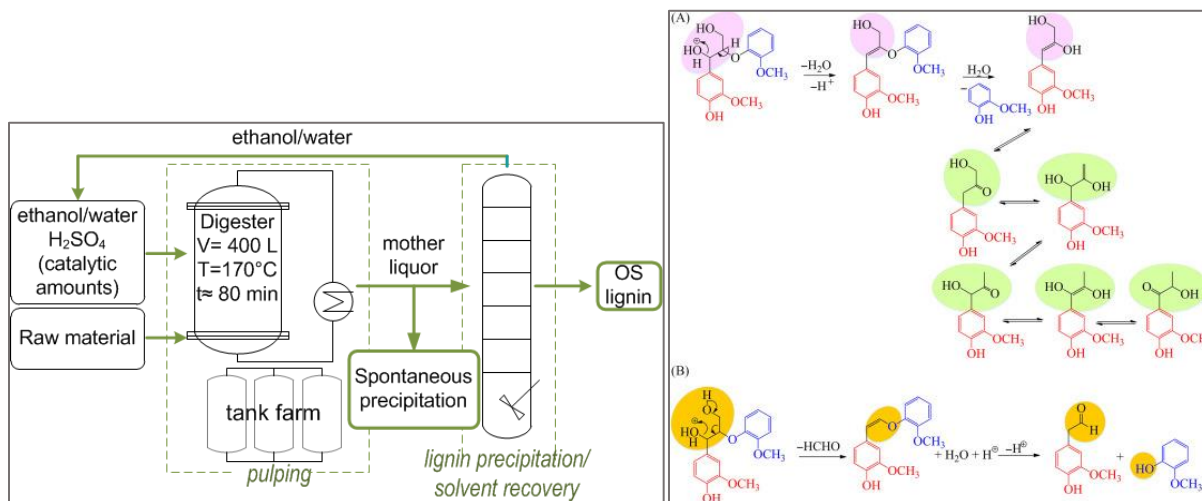


Figure A. 6. Organosolv pulping process and the chemistry involved. Adapted from Rossberg et al. (2019).<sup>99</sup>

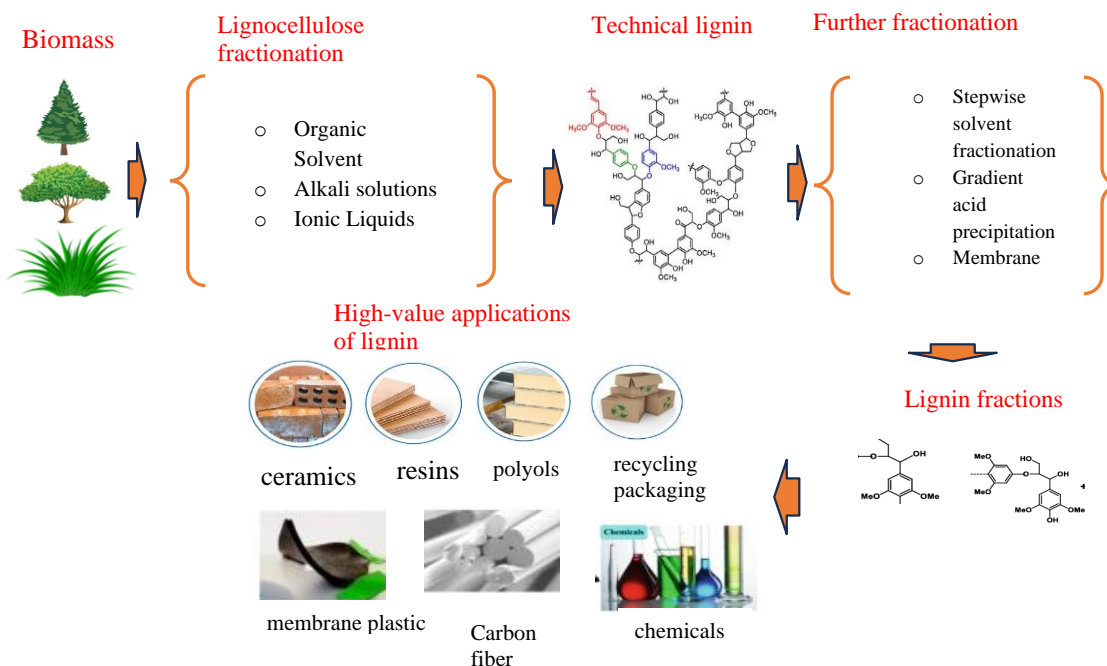


Figure A. 7. Schematic view of the utilization pathways of lignin in a biorefinery. Reproduced from Xu et al. (2020).<sup>100</sup>

#### 4.2.1.3 Applications of Lignin

Lignin was formerly frequently thought of as a waste by-product of bio-ethanol industries and paper pulping mills. It is typically used in recovery boilers in these industries to generate heat and power<sup>1,11,101–103</sup> with less than 1% of lignin being utilized as filler in paints, elastomeric matrices, ink varnishes, and other applications, as well as surfactants and dispersants.<sup>30</sup> The primary reason for this is the intrinsic structural heterogeneity and diversity found in lignin's composition. But in the last few years, there has been a surge in interest in using lignin as a useful feedstock to produce bio-based polymeric materials (resins and polymers) and bio-aromatic compounds (such as vanillin and phenols)<sup>104</sup> Due to its availability, the presence of different functional groups (hydroxyl and aromatic) on it, its lipophilic-hydrophilic structure, biodegradability, antioxidant properties, and ability to reinforce, lignin is a promising material for producing carbon fibers, bio-based polymeric materials (resins and polymers), and bio-aromatic chemicals (like vanillin and phenols) as shown in Figure A.7. Lignin is utilized as a dispersant or reinforcement filler in thermoplastic polymers.<sup>80,105</sup> Chettri et al., (2023); Mohammadinejad et al., (2019); Roy et al., (2020); Spiridon, (2020) have reviewed the application of lignin for biofuel production, and sustainable agricultural product production such as lignin-coated fertilizers, and biopesticides. Thielemans et al. (2002) proposed novel applications for vinyl esters and thermosetting polyesters in their investigation of lignin's solubility in resin systems. At 20°C, they observed that lignin acting as a plasticizer increased the glass-transition temperature and decreased the modulus of the thermosetting polyesters. Adding double bonds made lignin more compatible with resin matrices, facilitating chemical integration by free-radical polymerization. This alteration improved lignin's solubility and absorption in resins containing styrene. Because lignin has an affinity for cellulosic fibers, it has also been used to reinforce natural fibers in composites.<sup>109</sup>

Kaushik et al. (2014) reported the application of lignin as a binder in glass wool building insulation. When lignin, an ammonium sulfate from Kraft paper manufacture, is sprayed on hot glass, it functions as a binder in glass wool insulation, promoting fiber binding.<sup>110</sup> Owing to their ability to bond, lignosulfonates are essential to produce biodegradable plastics, plywood, ceramics, carbon black, animal feed pellets, fiberglass insulation, fertilizers, herbicides, linoleum paste, and soil stabilizers. Reasonably strong composites at an inexpensive price are produced with this binder. The mechanical properties such as toughness, tensile strength of particle boards, plywood, hardboard, and other panel boards are improved when lignin-based modifiers are added to formaldehyde-based binders. Lignin has also found its use in batteries. It has been reported to be essential for improving energy storage devices' functionality. Without affecting the properties of the graphite powder itself, it forms a thin layer on its surface to effectively avoid a drop in overvoltage.<sup>110</sup> In the food industries, potential applications for lignin include adding alkali lignin as a roughage or fiber source to pet and human food. A high dietary fiber consumption is associated with a lower incidence of colon cancer, according to a large body of research in this area.<sup>111</sup> In cement, it has been discovered that using low quantities of lignin and modified lignin strengthens high-performance concrete and lessens the harm that moisture and acid rain may do to external walls. Additionally, some specific lignin can increase cement pastes' compressive strength.<sup>110</sup> Even though lignin has promising potential as an alternative to fossil-based products, it is highly underutilized. The structure of lignin is complex with different variations based on plant species, isolation methods, process conditions, etc. Structural heterogeneity affects the physicochemical properties of lignin which in turn hinders its application in materials where constant properties are required. It is therefore important to understand the structure of lignin for its further utilization in chemicals and materials.<sup>106</sup> The simplest and most efficient way to reduce

the structural heterogeneity of lignin by producing lignin fractions with desired properties is to further fractionate technical lignin.

## APPENDIX B

### DES Preparation and Lignin Fractionation Procedures and Images

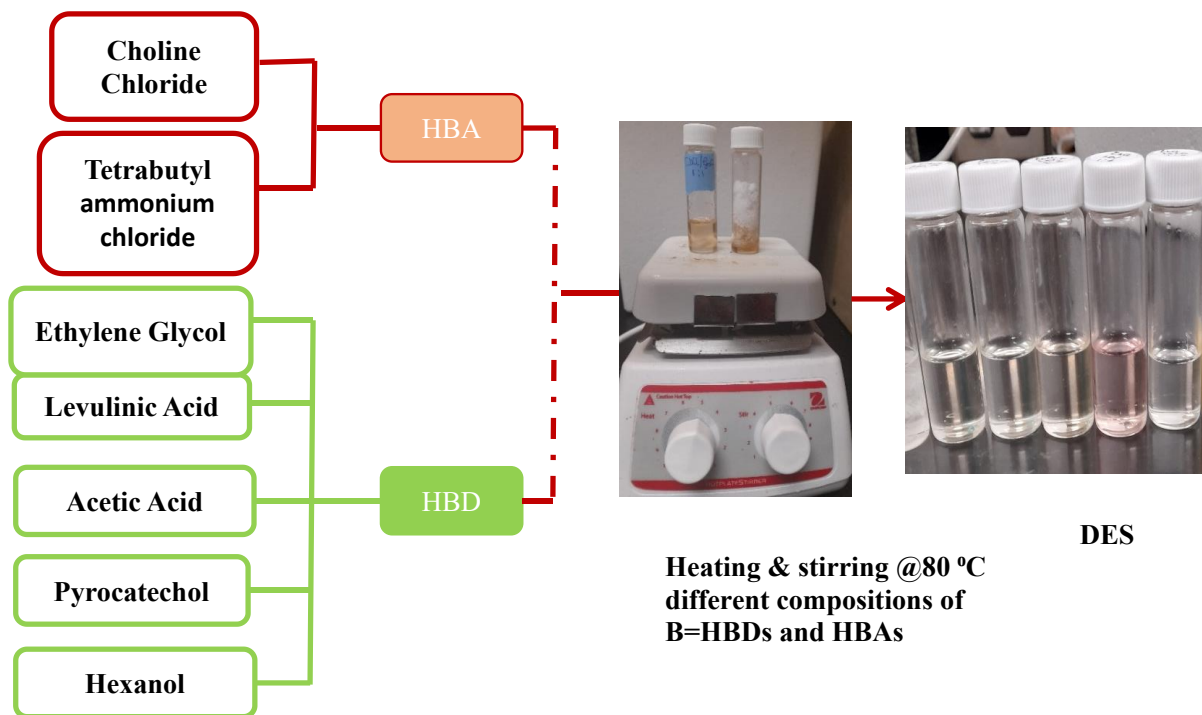


Figure B. 1. DES Preparation method.

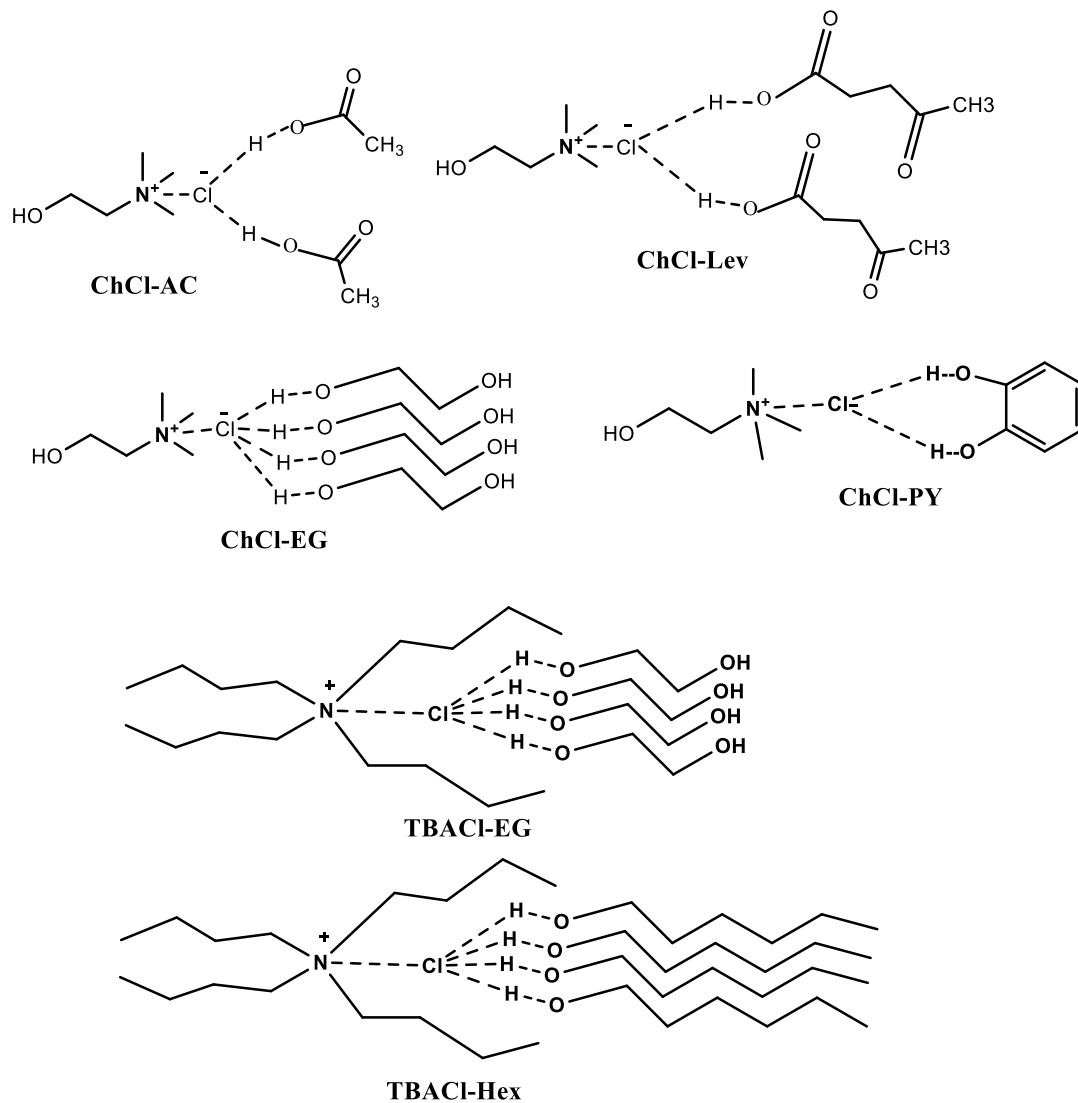


Figure B. 2. Chemical Structures of the seven formulated DES.



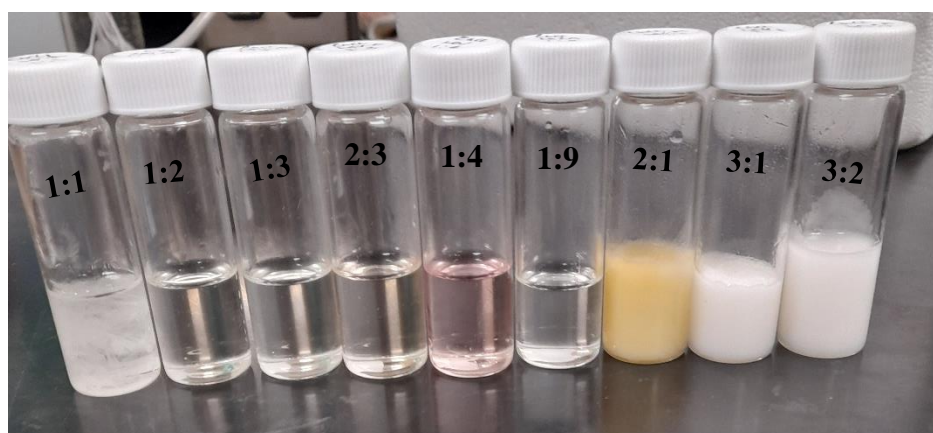
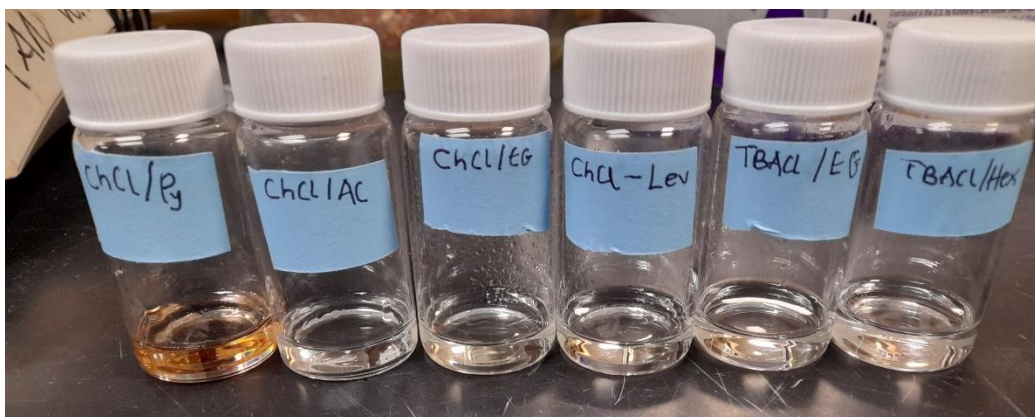


Figure B. 3. Images of some designed DES.

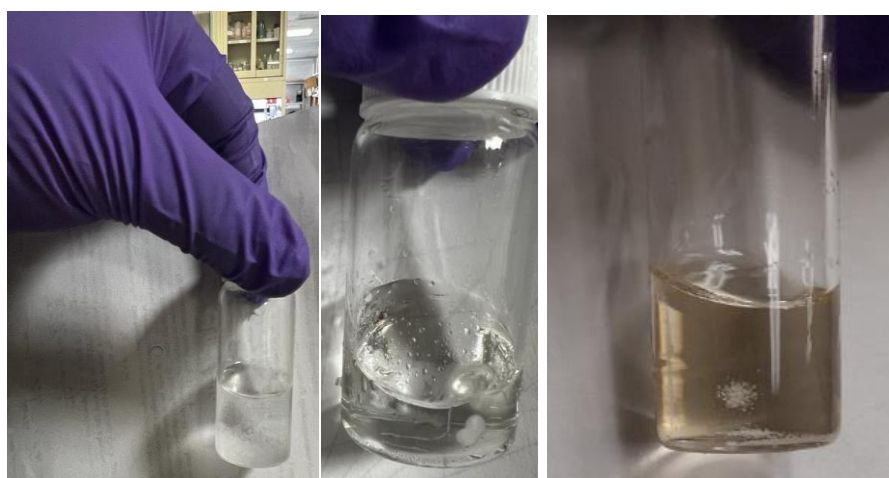
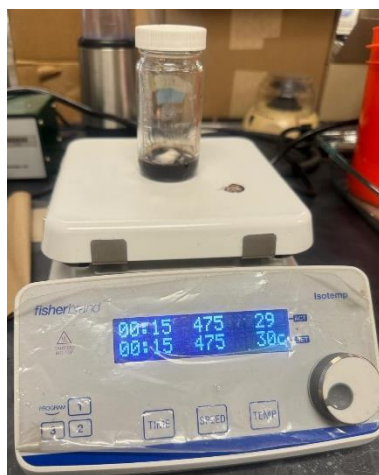


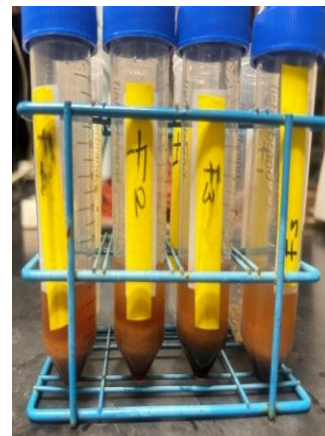
Figure B. 4. Images of different freezing points of designed DES.



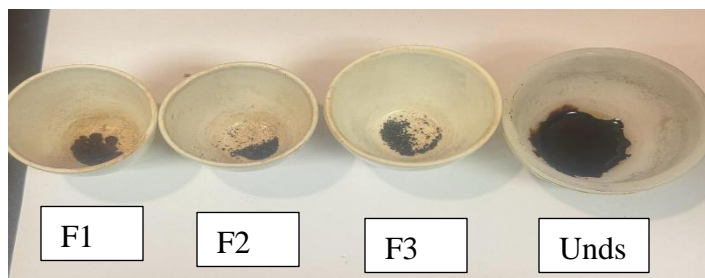
Lignin fractionation set-up



Vacuum filtration



Precipitation & Centrifugation



DI water precipitated fractions.



Lignin fractions before air-drying.



Acetone precipitated fractions

Figure B. 5. Images of different precipitated lignin fractions.

### **4.3. Biography**

Gloria Agyapong, a first-generation graduate student from Ghana graduated from the University of Cape Coast in 2020 with a Bachelor of Science degree in Industrial Chemistry. Her love for chemical engineering processes, especially those involving sustainable materials, was ignited by her high school studies in General Science at Chemu Senior High School, Ghana. After receiving her undergraduate degree, Gloria pursued her dream of studying abroad while serving as a teaching assistant in the University of Cape Coast's chemistry department for a year as part of her mandatory national duty.

She was admitted to graduate school at the University of Maine in 2022 to pursue a Master of Science degree in Chemical Engineering. Her research concentrates on developing cost-effective environmentally friendly solvents, called Deep Eutectic Solvents, for efficient lignin fractionation and currently working on her first publication. Her work aims to reduce the structural heterogeneity of lignin, producing fractions suitable for high-value applications. Gloria is interested in lignin chemistry, renewable materials, biomass conversion, and sustainable materials. Following the defense of her master's thesis, Gloria plans to pursue a Ph.D. in chemical engineering. Outside of school, she takes comfort in volunteering at her church, loves to hang out with friends, and sometimes just naps when she needs one. Gloria is a candidate for the Master of Science degree in Chemical Engineering from the University of Maine in August 2024.