Physico-Chemical Characterization of Lignin Isolated from Industrial Sources for Advanced Applications

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Faculty of Forestry
University of Toronto

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Abstract

Lignin is generated in large quantities as by-products in pulping industries and biorefineries through various processes. Lignin is currently isolated from the by-products but its application is limited due to non-uniform structure and unique chemical reactivity. However, advanced pulping industries and biorefineries involve improvement of the value derived from their lignin-containing by-product by converting them into new, advanced and high-value added products. This endeavor not only improves resources but also return revenue to their operations. The important strategy in this research is the isolation of lignin from pulping industry and biorefinery by-products and its further conversion into advance products such as microspheres. The specific objective was to investigate the physico-chemical and thermal characteristics of isolated lignin as well as fundamental studies of lignin solubilization in different organic solvents for the synthesis of lignin microspheres. The physico-chemical properties and the thermal behavior of lignin samples were characterized by using different analytical and thermal techniques. The solubilities of lignin samples were determined in different organic solvents and compared with the computed solubility parameter. For synthesis of lignin microspheres, either lignin was modified to lignin acetate to improve its solubility or the the soluble part of lignin in organic solvent was used in the process. The results showed that the molecular structure, functional groups, molecular weight, glass transition temperature and onset decomposition temperature of isolated lignins depend on the extraction
process and plant source. Solubilities of lignins isolated from different sources vary in different organic solvents. However, the solubility of lignin in organic solvents is not predictable due to poor correlation between the solubilities of lignins and their solubility parameters. Uniform lignin acetate microspheres were synthesized with a size of about 1 μm and narrow size distribution by using dichloromethane independent on the lignin source. Ethyl acetate was found as an alternative organic solvent useful in preparing lignin microspheres, which has relatively lower toxicity to human and the environment than dichloromethane. Finally, uniform lignin microspheres were synthesized from solubilized parts of two industrial lignins (hardwood kraft and non-wood soda lignins) in ethyl acetate under controlled conditions which have not been reported before.
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# Contents

Abstract ............................................................................................................................ ii

Acknowledgments .............................................................................................................. iv

List of Tables ...................................................................................................................... xi

List of Figures .................................................................................................................... xiv

List of Abbreviations ....................................................................................................... xviii

Nomenclature ..................................................................................................................... xx

CHAPTER 1 Introduction ...................................................................................................... 1

1.1 Background and motivation ......................................................................................... 1

1.2 Hypothesis .................................................................................................................. 3

1.3 Research objectives .................................................................................................... 3

1.4 Thesis outline ............................................................................................................. 4

CHAPTER 2 Literature Review .......................................................................................... 6

2.1 Wood components ..................................................................................................... 6

2.2 Lignin chemistry ....................................................................................................... 9

2.2.1 Reactive functional groups in lignin ........................................................................ 14

2.2.2 Lignin-Carbohydrate Complex (LCC) .................................................................... 14

2.3 Delignification of lignocellulosic biomass .................................................................. 16

2.3.1 Wood pulping ........................................................................................................ 16

2.3.2 The biorefinery ..................................................................................................... 19

2.4 Isolation methods for lignin recovery ......................................................................... 20

2.5 Industrial lignins ....................................................................................................... 21

2.5.1 Kraft lignin ............................................................................................................ 22

2.5.2 Soda lignin ............................................................................................................. 22
2.5.3 Lignosulfonate (Sulfite lignin) ................................................................. 24
2.5.4 Organosolv Lignin ........................................................................ 24
2.5.5 Lignin from Hydrolysis (Biomass conversion techniques) .......... 25
2.6 Current uses of lignin for value-added product ................................. 28
  2.6.1 Kraft Lignin .................................................................................. 29
  2.6.2 Lignosulfonates .......................................................................... 31
  2.6.3 Non-Sulfonated Industrial Lignins ................................................. 31
2.7 Advanced applications of lignin ........................................................ 32
  2.7.1 Lignin-based micro/nanoporous materials .................................... 32
  2.7.2 Lignin nanotubes ........................................................................ 33
  2.7.3 Lignin nanofibers ........................................................................ 33
  2.7.6 Lignin micro/nanoparticles .......................................................... 36
CHAPTER 3 Characterization of lignins isolated from steam exploded residues and kraft black liquor ............................................................. 38
  3.1 Introduction ....................................................................................... 38
  3.2 Experimental ................................................................................... 39
    3.2.1 Lignin samples and lignin isolation processes ......................... 39
    3.2.2 Characterization methods ......................................................... 42
  3.2 Results and discussion .................................................................... 49
    3.2.3 The yield percentage of isolated Lignin .................................... 49
    3.2.4 Optical microscopic images of isolated lignins and their origins .... 49
    3.2.5 Analysis of lignin ..................................................................... 51
    3.2.6 Bulk Density of lignin samples ............................................... 52
    3.2.7 FTIR Spectroscopy ................................................................. 52
    3.2.8 Elemental composition of lignin .............................................. 57
CHAPTER 3 Physical Characteristics of Lignin from Different Sources ........................................... 53

3.2.9 Heating value estimation ........................................................................................................ 58
3.2.10 Total hydroxyl and carboxyl content ....................................................................................... 61
3.2.11 Determination of G/H/S ratio by $^{31}$PNMR ..................................................................... 63
3.2.12 $^1$H-NMR spectrometry ...................................................................................................... 64
3.2.13 Double bonds equivalent (DBE) ......................................................................................... 68
3.2.14 Solubility of lignin in alkaline solution .................................................................................. 70
3.2.15 Determination of Molecular Mass Distribution (MMD) of lignin ...................................... 71
3.2.16 Potential applications for lignin from different sources ....................................................... 76
3.3 Conclusions ................................................................................................................................. 80

CHAPTER 4 Thermal Characteristics of Lignin Residue from Industrial Processes .... 81

4.1 Introduction ................................................................................................................................. 81
4.2 Experimental ............................................................................................................................... 83
4.2.1 Materials ................................................................................................................................. 83
4.2.2 Lignin Isolation ....................................................................................................................... 83
4.2.3 Ash Content Determination .................................................................................................... 84
4.2.4 Energy-Dispersive X-Ray Spectroscopy (EDS) .................................................................... 84
4.2.5 X-ray Diffraction (XRD) ....................................................................................................... 84
4.2.6 Thermogravimetric Analysis (TGA) ..................................................................................... 85
4.2.7 Differential Scanning Calorimetry (DSC) ............................................................................. 85
4.3 Results and discussion ............................................................................................................... 85
4.3.1 Ash Analysis ........................................................................................................................... 85
4.3.2 Thermogravimetry Analysis ................................................................................................ 89
4.3.3 Glass Transition ..................................................................................................................... 92
4.4 Conclusions ............................................................................................................................... 94

CHAPTER 5 Solubility of lignin and lignin acetate in organic solvents ........................................... 96
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Lignin dissolution behavior</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Solubility parameters</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Thermodynamics background</td>
</tr>
<tr>
<td>5.1.4</td>
<td>Solubility parameter theory</td>
</tr>
<tr>
<td>5.1.5</td>
<td>Estimation of solubility parameters (Group contribution methods)</td>
</tr>
<tr>
<td>5.2</td>
<td>Experimental</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Materials</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Acetylation of lignin</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Solubility determination of lignin in different organic solvents</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Determination of hydroxyl content using $^{31}$PNMR</td>
</tr>
<tr>
<td>5.2.5</td>
<td>Molecular weight determination using HPSEC</td>
</tr>
<tr>
<td>5.3</td>
<td>Results and discussion</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Computing δ-value of lignin and lignin acetate based on the expanded C9</td>
</tr>
<tr>
<td></td>
<td>formula</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Solubility of lignins from different sources in organic solvents</td>
</tr>
<tr>
<td>5.3.3</td>
<td>The effect of lignin molecular weight on the solubility</td>
</tr>
<tr>
<td>5.3.4</td>
<td>Solubility of acetylated lignins in organic solvents</td>
</tr>
<tr>
<td>5.3.5</td>
<td>Solubility of lignin in ethyl acetate</td>
</tr>
<tr>
<td>5.4</td>
<td>Conclusions</td>
</tr>
<tr>
<td>6.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>6.1.1</td>
<td>Methods for synthesis of micro/nanoparticles</td>
</tr>
<tr>
<td>6.1.5</td>
<td>Surfactant choice</td>
</tr>
<tr>
<td>6.1.6</td>
<td>Overview of Polyvinyl Alcohol (PVA)</td>
</tr>
</tbody>
</table>
6.1.7 Adhesion of PVA on the surface of particles.................................................. 129
6.1.8 Variables........................................................................................................... 130
6.1.9 Theory of microspheres formation .................................................................. 130
6.1.10 Synthesis of hollow spheres ......................................................................... 133
6.1.11 Dynamic Light Scattering techniques ......................................................... 133
6.1.12 Surface Charge (Zeta Potential) ................................................................... 134

6.2 Experimental ....................................................................................................... 136
6.2.1 Materials .......................................................................................................... 136
6.2.2 Acetylation of lignin ......................................................................................... 137
6.2.3 Synthesis of lignin acetate microspheres at different conditions .......... 137
6.2.4 Preparation of lignin microspheres and lignin acetate microspheres using different lignins ........................................................................................................ 138
6.2.5 Preparation of lignin acetate hollow spheres .............................................. 139
6.2.6 Determination of size and size distribution of lignin particles by using Dynamic light scattering (DLS) technique ................................................................. 141
6.2.7 Determination of zeta potential by using DLS technique ......................... 141
6.2.8 Morphology of lignin particles by Scanning Electron Microscopy ...... 141
6.2.9 The yield percentage determination ............................................................... 141
6.2.10 Mixture Stability test .................................................................................... 142

6.3 Results and discussion ......................................................................................... 142
6.3.1 The effect of preparation parameters on the lignin particles formation .... 142
6.3.1.1 The influence of mixing shear rate .............................................................. 142
6.3.1.2 Formation of lignin acetate hollow spheres ............................................ 147
6.3.1.3 The role of surfactant in microspheres formation .................................. 149
6.3.1.4 The influence of surfactant concentration .............................................. 150
6.3.1.5 The influence of mixing time ................................................................. 154
6.3.1.6 The effect of organic solvent on the particle formation .......................... 156
6.3.1.7 Stability of the lignin acetate microspheres suspension ....................... 160
6.3.2 Synthesis and characterization of lignin acetate microspheres from different sources 163
   6.3.2.1 Synthesis of lignin acetate microspheres in DCM............................. 163
   6.3.2.2 Synthesis of lignin acetate microspheres in EA............................. 167
   6.3.2.3 Preparation and characterization of lignin microspheres in EA........... 171
   6.3.2.4 Yield percentages of microspheres .............................................. 174
   6.3.2.5 Stability of the lignin microspheres suspension ............................. 175
   6.3.2.6 The effect of the Mw and number of hydroxyl groups on the size of the lignin microspheres ................................................................. 178
6.4 Conclusions............................................................................................. 179

CHAPTER 7 Final conclusions and future work ............................................. 180
   7.1 Summary and conclusions ..................................................................... 180
   7.2 Contributions........................................................................................ 181
   7.3 Future work ......................................................................................... 182
References..................................................................................................... 183
List of Tables

Table 1. Chemical composition of different wood and agricultural residues (% w/w), Percentage based on dry weight ................................................................................................................. 8
Table 2. Different types of linkages between phenylpropanoid units in lignin as percent of total linkages (Sjöström, 1993) .................................................................................................................................................. 12
Table 3. A comparison between the properties of wheat straw and hemp soda lignins (Lora and Glasser, 2002) ...................................................................................................................................................... 23
Table 4. Typical properties of lignins isolated from steam explosion process (Lora and Glasser, 2002) ......................................................................................................................................................... 25
Table 5. Chemical composition of the industrial lignins (Vishtal and Kraslawski, 2011a) .................................................................................................................................................................. 27
Table 6. Original source of lignin samples .................................................................................................................................................................................. 41
Table 7. Composition of the four lignin samples ................................................................................................................................................................. 51
Table 8. Fourier transform infrared of four lignin samples ................................................................................................................................................ 56
Table 9. Summary of important bands .................................................................................................................................................................................. 57
Table 10. Elemental composition, empirical formula, higher heating value (HHV) and H/C ratio of lignin samples .................................................................................................................................................. 60
Table 11. Data obtained for the total hydroxyl and carboxyl content with titration and $^{31}$P-NMR, and total phenolic and aliphatic hydroxyl contents with $^{31}$P-NMR (unit mmol/g) .................................................................................................................................................. 62
Table 12. Contents of lignin unit percentage in lignin samples obtained from $^{31}$PNMR spectra .................................................................................................................................................................. 64
Table 13. Area and number of hydrogen in lignin samples obtained from 1H-NMR spectrums .................................................................................................................. 66
Table 14. C9-formula, expanded C9-formula, double bond equivalent (DBE) and molecular weight for lignin samples .................................................................................................................. 69
Table 15. The number average (Mn), weight average (Mw) molecular weight, polydispersity (PD), number average (Dpn) and mass average (Dpw) degree of polymerization for the four lignin samples .......................................................................................................................... 76
Table 16. Summarized physico-chemical properties of lignin samples with potential applications ........................................................................................................................................ 79
Table 17. Ash content and moisture content of lignin samples .................................................. 87
Table 18. Onset temperature, degradation temperature, and percentage of charred residues of original and isolated lignins .................................................................................................................. 92
Table 19. Onset temperature and glass transition temperature for original and isolated lignin samples ........................................................................................................................................ 94
Table 20. Values of $\Delta e_i$ and $\Delta v_i$ for atoms and groups in lignin (Fedors, 1974; Ni and Hu, 1995) ........................................................................................................................................................................... 104
Table 21. Calculated $\Delta e_i$ and $\Delta v_i$ for each lignin based on the number of the functional group and the ratio of G/S/H (*$\Delta v_i$ is the correction factor for divergence in the v value (Ni and Hu, 1995)) .......................................................................................................................................................................................... 106
Table 22. Calculated $\Delta e_i$ and $\Delta v_i$ for each lignin acetate based on the number of the functional group and the ratio of G/S/H (*$\Delta v_i$ is the correction factor for divergence in the v value) ........................................................................................................................................................................... 108
Table 23. $\delta$–value (from Hildebrand theory) and $\delta_h$-value (from Hansen theory) of organic solvents and water (Hansen, 2000; Hildebrand and Scott, 1950) ...................... 109
Table 24. The number average (Mn), weight average (Mw), peak average (Mp) molecular weights and polydispersity (PD) of soluble and insoluble part of lignin in ethyl acetate (EA) ....................................................................................................................................................... 122
Table 25. Stability of colloids in relationship to the particle charge ......................................... 136
Table 26. Preparation parameters for synthesis of lignin acetate microspheres ............ 138
Table 27. Minimum, maximum and mean particle size prepared by using magnetic stirrer and measure by imageJ software .................................................................................................................................................. 143
Table 28. Average size, PDI and zeta-potential of lignin acetate microspheres in EA and DCM .................................................................................................................................................................. 159
Table 29. Some physical properties of selected organic solvent (Patil et al., 2007; Sah, 1997) ........................................................................................................................................................................... 160
Table 30. Average size, PDI and zeta-potential of lignin acetate microspheres prepared in dichloromethane and ethyl acetate subjected to stability test at room temperature over time. Mean value (±Standard Deviation) ............................................................................................................................................... 162
Table 31. Reproducibility of lignin microspheres by using controlled parameters through emulsion solvent evaporation technique ................................................................. 164
Table 32. Average particle size and polydispersity index of lignin acetate microspheres prepared in DCM ........................................................................................................... 166
Table 33. Average size and PDI of lignin acetate microspheres, EA was used as organic solvent .......................................................................................................................... 169
Table 34. Solubility of lignin in DCM and EA ........................................................................ 171
Table 35. Average size and PDI of lignin acetate microspheres, EA was used as organic solvent .......................................................................................................................... 172
Table 36. The yield percentage of the particles .................................................................. 175
Table 37. The average size of microspheres in the first day and after 60 days in aqueous suspension. P-values is for Z-Ave of the lignin microspheres ................. 176
List of Figures

Figure 1. Structural units of lignin. Adapted from (Adler, 1977; Pettersen, 1984)............... 9
Figure 2. The most common inter-monomeric linkages between lignin units. Adapted from (Karhunen et al., 1995; Sjöström, 1993) ........................................................................................................ 11
Figure 3. Schematic representation of the lignin structure suggested (Adler, 1977). Adapted from (Tejado et al., 2007) .................................................................................................................. 13
Figure 4. Lignin–carbohydrate complex in grass involving ferulic acid (Adapted from Brandt et al., 2013) .................................................................................................................. 15
Figure 5. Synthetic of lignin nano-containers by inverse mini-emulsion (with permission from (Yiamsawas et al., 2014) ........................................................................................................ 35
Figure 6. Processes for lignin isolation ...................................................................................... 42
Figure 7. The reaction of lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) for quantitative $^{31}$P NMR analysis (Adopted from Yáñez-S et al., 2014) ........................................................................................................ 48
Figure 8. Microscopic images of isolated lignins and their original source. The scale bar is 0.5mm (NA; not available) ........................................................................................................ 50
Figure 9. UV spectrum of acid soluble lignins .......................................................................... 52
Figure 10. Comparison between the raw materials and their isolated lignin; L1 (isolated from bioethanol biorefinery residue) and L2 (isolated from kraft black liquor) ............... 53
Figure 11. FTIR Spectra of isolated lignin samples in the range of (a) 4000-600cm$^{-1}$ and (b) 1400–1000 cm$^{-1}$ ........................................................................................................ 55
Figure 12. $^{31}$P-NMR spectra of four lignin samples; Lignin units are syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H) .................................................................................. 63
Figure 13. $^1$H-NMR spectra of four acetylated lignins .............................................................. 65
Figure 14. Solubility of lignin samples in NaOH ...................................................................... 70
Figure 15. Molecular weight distributions of standards; Sodium poly(styrene sulfonates) (PSS) with different peak molecular weight of 1100, 1830, 4230 and 6520 daltons ...... 71
Figure 16. Calibration curve for the PSS standard solutions ...................................................... 72
Figure 17. Molecular weight distributions of lignin samples, a) Intensity vs. Time b) $A_i$ (mass fraction) vs. $M_i$ (Molecular weight) ................................................................................ 73
Figure 18. EDS analysis of lignin ash ................................................................. 88
Figure 19. XRD spectra of lignin ash ................................................................. 89
Figure 20. TGA thermogram of original and isolated lignin samples ................. 91
Figure 21. DSC curves of lignin samples .......................................................... 93
Figure 22. Typical repeating units of lignin (Quesada-Medina et al., 2010) .......... 104
Figure 23. Solubility of 100 mg lignin in 10 mL of different organic solvents ...... 112
Figure 24. The relationship between solubility and weight average molar mass of lignin samples (L1-L4) ................................................................. 114
Figure 25. Solubility of 100 mg of acetylated lignin in 10 ml of different organic solvents ................................................................. 115
Figure 26. 31PNMR spectrum of lignin samples and their soluble part in EA........ 117
Figure 27. Phenolic hydroxyl and aliphatic hydroxyl content of lignin and soluble part of lignin in EA ................................................................. 118
Figure 28. Calibration curve of the PSS standards for molecular weight determination of a) insoluble part of lignins in EA and b) soluble part of lignins in EA .................. 119
Figure 29. Molar mass distributions of soluble and insoluble lignin samples in ethyl acetate .............................................................................. 120
Figure 30. Comparison between the molar mass distributions of soluble part of lignin samples in EA .................................................................................. 121
Figure 31. Mw of soluble and insoluble part of lignin in EA ............................... 123
Figure 32. The process for lignin microsphere formation A) lignin in organic phase and surfactant in aqueous phase, B) Intermix the system, C) Solvent evaporation D) Solidification .............................................................................. 127
Figure 33. Chemical structure of Polyvinyl alcohol (PVA) .................................. 129
Figure 34. PVA interactions at the surface of PLGA nanoparticles (with permission from Murakami et al., 1999) ................................................................. 129
Figure 35. Schematic of solvent diffusion and evaporation steps ...................... 131
Figure 36. Schematic of mass transfers of solvent during solidification of microsphere .............................................................................. 132
Figure 37. Method for synthesis of lignin microspheres: (1) lignin-containing organic solvent is mixed with an aqueous PVA solution to make an oil-in-water emulsion, (2)
Diffusion of organic solvent from organic phase to aqueous phase, (3) washing and collection the particles, and finally (4) drying samples by using freeze drier. .......... 140

Figure 38. Particle size distribution of lignin acetate microspheres at different shear rate; Particle size was determined by a) imageJ software and b) DLS technique .......... 143

Figure 39. Average particle size (Z-Ave) and polydispersity (PDI) of lignin acetate microspheres at different shear rate applied by homogenizer ............................................. 144

Figure 40. SEM images of lignin acetate microspheres which prepared by using magnetic stirrer at low shear (800 rpm and 1000 rpm) and homogenizer at high shear rate (10,000-20,000 rpm). ............................................................................................................. 145

Figure 41. The relationship between the diameter of the agitator (D) and the agitation rate (N) with the maximum size of the lignin acetate microspheres (d_{max}) .......... 147

Figure 42. SEM images of lignin acetate hollow spheres ........................................................................ 148

Figure 43. Particle size distribution of lignin acetate hollow sphere ............................................. 148

Figure 44. Formation of lignin microspheres and hollow spheres at different shear rate .................................................................................................................. 149

Figure 45. A schematic of surfactant stabilized lignin acetate microsphere and lignin acetate hollow sphere ......................................................................................... 150

Figure 46. Particle size distributions of lignin acetate microspheres at different PVA concentration .................................................................................................................. 151

Figure 47. Average particle size and PDI of lignin acetate microspheres at different PVA concentration .................................................................................................................. 152

Figure 48. SEM images of lignin acetate microspheres at different PVA concentration (0.0-2.0%). Agitation rate was 10,000 rpm for all cases, unless stated on the image . 153

Figure 49. Particle size distribution of lignin acetate microspheres at different time for agitation .................................................................................................................. 154

Figure 50. Average particle size and polydispersity index (PDI) of lignin acetate microspheres at different mixing time ........................................................................... 155

Figure 51. SEM images of lignin acetate microspheres at different mixing time (the scale bar is 5 μm) ............................................................................................................. 156

Figure 52. SEM images of lignin acetate microspheres at different organic solvents (the scale bar is 5 μm) ............................................................................................................. 157
Figure 53. Particle size distribution of lignin acetate microspheres with different organic solvents.................................................................................................................................................................................. 158

Figure 54. Lignin acetate microspheres after 60 days in neutral suspension (the scale bar is 10 \( \mu \text{m} \))........................................................................................................................................................................................................................................... 163

Figure 55. Comparison between lignin acetate microspheres prepared with the same conditions........................................................................................................................................................................................................................................................................................................... 164

Figure 56. SEM images of lignin acetate microspheres when DCM was chosen as organic solvent in the method (the scale bar is 5 \( \mu \text{m} \))........................................................................................................................................................................................................................................................................................................... 165

Figure 57. Particle size distributions of lignin acetate microspheres isolated from different sources........................................................................................................................................................................................................................................................................................................... 166

Figure 58. Zeta potential of lignin acetate microspheres ........................................................................................................................................................................................................................................................................................................... 167

Figure 59. SEM micrographs of lignin acetate microspheres when EA was chosen as organic solvent in the method (the scale bar is 5 \( \mu \text{m} \))........................................................................................................................................................................................................................................................................................................... 168

Figure 60. Size distribution of lignin acetate when EA was used as dispersing solvent ........................................................................................................................................................................................................................................................................................................... 169

Figure 61. Zeta potential of lignin acetate microspheres in EA ........................................................................................................................................................................................................................................................................................................... 170

Figure 62. SEM micrographs of lignin microspheres. EA was chosen as organic solvent in the method (the scale bar is 5 \( \mu \text{m} \))........................................................................................................................................................................................................................................................................................................... 172

Figure 63. Size distribution of lignin microspheres when EA was used as dispersing solvent ........................................................................................................................................................................................................................................................................................................... 173

Figure 64. Zeta potential of lignin microspheres (L3 was rejected by the DLS analysis) ........................................................................................................................................................................................................................................................................................................... 174

Figure 65. SEM images of lignin acetate microspheres after 60 days in 0.1% aqueous suspension (arrow shows the agglomerations) (the scale bar is 5 \( \mu \text{m} \))........................................................................................................................................................................................................................................................................................................... 177

Figure 66. SEM images of lignin microspheres after 60 days in 0.1% aqueous suspension (arrow shows the agglomeration) (the scale bar is 5 \( \mu \text{m} \))........................................................................................................................................................................................................................................................................................................... 178
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$HNMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>$^{31}$PNMR</td>
<td>Phosphorous Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>ACE</td>
<td>Acetone</td>
</tr>
<tr>
<td>ACL1</td>
<td>Acetylated lignin isolated from Bioethanol biorefinery residue</td>
</tr>
<tr>
<td>ACL2</td>
<td>Acetylated lignin isolated from kraft process</td>
</tr>
<tr>
<td>ACL3</td>
<td>Acetylated commercial softwood kraft lignin</td>
</tr>
<tr>
<td>ACL4</td>
<td>Acetylated commercial non-wood soda lignin</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerization</td>
</tr>
<tr>
<td>Bp</td>
<td>Boiling point</td>
</tr>
<tr>
<td>CED</td>
<td>Cohesive Energy Density</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
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<td>Number average degree of polymerization</td>
</tr>
<tr>
<td>Dpw</td>
<td>Mass average degree of polymerization</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EA</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersed X-ray</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fortier Transform Infrared</td>
</tr>
<tr>
<td>HHV</td>
<td>Higher heating value</td>
</tr>
<tr>
<td>ICDD</td>
<td>International Centre for Diffraction Data</td>
</tr>
<tr>
<td>KDa</td>
<td>Kilodaltons</td>
</tr>
<tr>
<td>L1</td>
<td>Lignin isolated from bioethanol biorefinery residue</td>
</tr>
<tr>
<td>L1-Orig</td>
<td>Bioethanol biorefinery residue</td>
</tr>
<tr>
<td>L2</td>
<td>Lignin isolated from black liquor</td>
</tr>
<tr>
<td>L2-Orig</td>
<td>Black liquor solid</td>
</tr>
<tr>
<td>L3</td>
<td>Commercial softwood kraft lignin</td>
</tr>
<tr>
<td>L3-I</td>
<td>Isolated lignin from commercial softwood kraft lignin</td>
</tr>
<tr>
<td>L4</td>
<td>Commercial non-wood soda lignin</td>
</tr>
</tbody>
</table>
L5  Commercial non-wood soda lignin
L5-I  Isolated lignin from commercial non-wood soda lignin
LCC  lignin-carbohydrate complex
LDV  Laser doppler velocimetry
Min  Minutes
$M_n$  Number-average molecular weight
$M_w$  Weight-average molecular weight
Na$_2$S  Sodium sulfide
NaOH  Sodium hydroxide
PD  Polydispersity
PDI  Polydispersity Index
PF  Phenol Formaldehyde
PLA  Polylactic acid
PLGA  Poly(lactic-co-glycolic acid)
PMMA  Poly(methyl methacrylate)
PNIPAM  Poly(N-isopropylacrylamide)
SEC  Size Exclusion Chromatography
SEM  Scanning Electron Microscopy
PSS  Poly(styrene sulfonate) sodium
TDI  Toluene diisocyanate
TGA  Thermogravimetry Analysis
THF  Tetrahydrofuran
TMDP  2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane
XRD  X-ray diffraction
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_a$</td>
<td></td>
<td>absolute absorbance</td>
</tr>
<tr>
<td>a</td>
<td>L/g.cm</td>
<td>absorptivity of the lignin</td>
</tr>
<tr>
<td>$A_{wa}$</td>
<td>m$^2$</td>
<td>surface area of water-air interface</td>
</tr>
<tr>
<td>A</td>
<td>ml</td>
<td>volume of sodium hydroxide solution for titration of acetylated lignin</td>
</tr>
<tr>
<td>$A_i$</td>
<td></td>
<td>slice area at each interval of molecular weight $M_i$</td>
</tr>
<tr>
<td>B</td>
<td>ml</td>
<td>volume of sodium hydroxide solution for titration of the blank</td>
</tr>
<tr>
<td>C</td>
<td>mmol/g</td>
<td>number of carboxyl groups</td>
</tr>
<tr>
<td>$C_1$</td>
<td></td>
<td>constant value</td>
</tr>
<tr>
<td>$C_s$</td>
<td>kg/m$^3$</td>
<td>concentration of solvent in the continuous phase</td>
</tr>
<tr>
<td>$C_{sol}$</td>
<td></td>
<td>solubility of solvent in the continuous phase</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td>dilution ratio</td>
</tr>
<tr>
<td>D</td>
<td>m</td>
<td>diameter of the agitator</td>
</tr>
<tr>
<td>$d_{max}$</td>
<td>m</td>
<td>largest drop size which can be formed under turbulence</td>
</tr>
<tr>
<td>E</td>
<td>cal/mol</td>
<td>cohesive energy</td>
</tr>
<tr>
<td>$h_i$</td>
<td></td>
<td>peak height at each interval of molecular weight $M_i$</td>
</tr>
<tr>
<td>K</td>
<td>m/s</td>
<td>evaporation constant</td>
</tr>
<tr>
<td>M</td>
<td>kg</td>
<td>total mass of solvent in the reactor</td>
</tr>
<tr>
<td>$M_i$</td>
<td>g/mol</td>
<td>molecular weight</td>
</tr>
<tr>
<td>N</td>
<td>eqv/L</td>
<td>normality of the sodium hydroxide solution</td>
</tr>
<tr>
<td>$N_i$</td>
<td>turns/s</td>
<td>agitation rate</td>
</tr>
<tr>
<td>$n_i$</td>
<td></td>
<td>number of molecules of molecular weight $M_i$</td>
</tr>
<tr>
<td>R</td>
<td>J/mol.K</td>
<td>gas constant</td>
</tr>
<tr>
<td>T</td>
<td>K</td>
<td>absolute temperature</td>
</tr>
<tr>
<td>t</td>
<td>s</td>
<td>time</td>
</tr>
<tr>
<td>V</td>
<td>ml</td>
<td>volume</td>
</tr>
<tr>
<td>$V_m$</td>
<td>cm$^3$/mol</td>
<td>molar volume</td>
</tr>
<tr>
<td>W</td>
<td>g</td>
<td>weight</td>
</tr>
<tr>
<td>$\delta$</td>
<td>(cal/cm$^3$)$^{1/2}$</td>
<td>Solubility parameter</td>
</tr>
<tr>
<td>$\Delta e_i$</td>
<td>cal/mol</td>
<td>atomic and group contributions for the energy of vaporization</td>
</tr>
<tr>
<td>$\Delta G_m$</td>
<td>J/mol</td>
<td>Gibbs free energy change on mixing</td>
</tr>
</tbody>
</table>
\( \delta_h \) (cal/cm\(^3\))\(^{1/2}\)  Solubility parameter (hydrogen bonding interactions)

\( \Delta H_m \) J/mol  enthalpy change on mixing

\( \Delta H_{vap} \) J/mol  enthalpy of vaporisation

\( \Delta S_m \) J/K.mol  entropy change on mixing

\( \Delta v_i \) cm\(^3\)/mol  atomic and group contributions for the molar volume

\( \rho_c \) kg/m\(^3\)  density of continuous phase

\( \sigma \) N/m  interfacial tension between continuous phase and dispersed phase
CHAPTER 1 Introduction

1.1 Background and motivation

The focus on biobased materials has increased the interest in lignin as a natural and sustainable source for manufacturing new biobased products. Lignin is produced in large quantities as by-product in pulping industries and biorefineries through different processes such as kraft, soda, organosolv, steam explosion, etc. In these industrial processes cellulosic fibers (in pulping industries) and hydrolyzed polysaccharides (in biorefineries) are extracted through depolymerization and/or derivatization of lignin. Lignin can be extracted from the residue however, the chemical structure of lignin alters depending on the industrial isolation procedures.

Lignin is currently burned in recovery boilers and only a small portion of lignin is used for value-added products (Lora and Glasser, 2002). The utilization of lignin for synthesis of advanced materials is very limited due to the unknown molecular structure of lignin and variations in molecular weight and functional groups. However, even with these drawbacks, the interest for developing lignin-based products is increasing because of the growing demand for sustainable products (Larry Hughes, 2014; Mousavioun and Doherty, 2010). For example, lignin is used in the synthesis of phenol formaldehyde resin due to existing the phenolic ring in the lignin macromolecule (Abdelwahab and Nassar, 2011; Alonso et al., 2004; Cheng et al., 2013; Khan et al., 2004b; Mankar et al., 2012; Sarkar and Adhikari, 2001a; Tejado et al., 2007; Zhang et al., 2013c). Lignin is also used as filler and reinforcing phase for polymer blends (Cazacu et al., 2004; Gosselink et al., 2004c; Hatakeyama et al., 2005; Kadla et al., 2002; Lora and Glasser, 2002; Reza Barzegari et al., 2012; Schorr et al., 2014). The high number of hydroxyl groups in lignin are an advantage for production of polyol through either direct utilization or after chemical modification for production of certain polymers such as polyurethane (Cateto et al., 2008; Huang and Zhang, 2002; Mahmood et al., 2013; Sarkar and Adhikari, 2001b).
Recently, new techniques have been exploited to develop more advanced materials from lignin with a controlled structure down to the micro or nano scale. For instance, lignin nano- or microparticles have been studied for their potential applications in different areas such as agricultural actives controlled release (Asrar and Ding, 2010; Chowdhury, 2014; Fernandez-Perez et al., 2011), food industry fat mimetics (Stewart et al., 2014), filler in composites (Jiang et al., 2013) and nano-sized coatings (Popa et al., 2011). Consequently, several methods has been developed for synthesis of lignin nano- or microparticles such as the solvent evaporation method (Asrar and Ding, 2010), carbonization (Gonugunta et al., 2012), adding hydrochloric acid to the solution of lignin in ethylene glycol (Frangville et al., 2012), adding non-solvent (water) to a solution of lignin acetate in tetrahydrofuran (Qian et al., 2014), locating lignin at the oil-water interface (Tortora et al., 2014), emulsifying the lignin aqueous solution in an organic phase of cyclohexane containing toluene diisocyanate and a surfactant (Yiamsawas et al., 2014) and polyaddition reaction of toluene diisocyanate with lignin in an inverse mini-emulsion (Wurm and Weiss, 2014).

The formulation of microparticles for agricultural actives controlled release have various advantages such as smaller dosage of agricultural actives, labour saving and safety, and less environmental impact. The technology for development of these formulations is costly due to the costs for the synthesis of biodegradable polymers (Wilkins, 1983). Using cheaper natural polymers such as lignin seems to be a viable way to overcome the high cost (Dubey et al., 2011). But it is important to note that the modification of lignin (i.e. acetylation) may increase the cost of the raw material. Also, lignin-based microparticles make the formulation economically viable due to dual function as a fertilizer and a as carrier for the agricultural actives. It should be note that the manufacturing of microspheres is easy with conventional equipments and it can be designed for a lignin-based matrix (Asrar and Ding, 2010).

It is important to add value to lignin derived as by-products in the pulping industry and biorefineries because it improves the economic feasibility of paper and biofuel production. Therefore, this thesis identified the type of impurities and the source of lignin from different industrial lignins which help to evaluate the lignin for different industrial
applications. Thermal properties of lignin isolated from different industrial residues were compared with their original sources. Physico-chemical properties of isolated lignins were determined based on their chemical structure. Solubility of lignin in a series of organic solvents was determined to identify the different degrees of solubility of lignins. Solubility parameters for each isolated lignin were calculated and applied based on the Hildebrand theory. This thesis also focused on producing uniform lignin-based microspheres by using the lignin isolated from different industrial sources.

1.2 Hypothesis

The main hypotheses of this work are:

- Isolated lignins from different origins and industrial processes have different thermal properties and chemical functionality.

- Solubility of lignin in organic solvents depends on the functional groups and molecular weight of the lignin macromolecule. The Hildebrand theory can be applied to explain the effect of lignin functional groups on the solubility of lignin in different organic solvents.

- Lignin has the potential to form into spherical microparticles through emulsion solvent evaporation technique if it can be solubilized in suitable organic solvents such as dichloromethane and ethyl acetate.

1.3 Research objectives

The main goal of this research is to investigate the fundamentals of solubilization and physico-chemical characteristics of lignin isolated from different industrial by-products for synthesis of lignin microspheres.
Objectives:

1. To physico-chemically characterize lignin isolated from kraft, soda and steam explosion processes.

2. To compare the thermal behavior of different industrial lignins before and after isolation process.

3. To determine the solubility of lignin in organic solvents in relation to the molecular weight and functional groups.

4. To synthesize lignin microspheres from either acetylated lignin or solubilized part of lignin in organic solvent.

1.4 Thesis outline

This thesis is divided into seven chapters: Introduction, literature review, four chapters that include the findings of this research, and a final chapter comprised of the recommendations and conclusions.

Chapter 2 contains the literature review on lignin chemistry, delignification processes and isolation methods in order to identify the contribution of lignin for current value-added products. This chapter also discusses the new micro/nanotechnologies that have been exploited to develop advanced materials from lignin such as lignin-based micro/nanoporous structures, nanotubes, nanofibers, micro/nanoparticles. In Chapter 3, the physico-chemical properties of isolated lignins are analyzed. Molecular structure, functional groups and molecular weights of two isolated lignins from different sources are compared with commercial lignins. In chapter 4, thermal behaviors of isolated lignins are compared with their original source and the impurities are identified from each source. In chapter 5, we discuss the solubilities of lignins isolated from different sources in a series of organic solvents. Solubility of lignin in organic solvents is evaluated by solubility parameter which is calculated based on the Hildebrand theory. Chapter 6 illustrates the effects of the preparation parameters on the formation of lignin
microspheres by using emulsion solvent evaporation technique. Lignin microspheres with different physical characteristics (size, size distribution and morphology) are synthesized by controlling the preparation parameters. The theories on the microspheres formation are reviewed in order to understand the mechanism of lignin microspheres formation during the micellization of the surfactant. Uniform lignin-based microspheres are successfully synthesized by either acetylation of lignin or by using the soluble part of lignin in ethyl acetate. To our knowledge, the synthesis of lignin microspheres (without acetylation) has never been studied before.
CHAPTER 2 Literature Review

2.1 Wood components

The three major components in wood are cellulose, hemicelluloses and lignin. Minor amount of organic extractives and inorganic materials are also present in wood. The percentage of each component depends on the source of the wood (Table 1). In different wood species their relative composition varies, and also the chemical composition of wood varies quantitatively among different tree types.

Cellulose is the main constituent in wood and the most common polymer in nature. The β-D-glucopyranose units are linked to each other through (1→4)-glucosidic ester bonds to build up the linear cellulose chains (Sjöström, 1993). The average degree of polymerisation for cellulose chain is in the range 8000 to 10000, which the network of cellulose makes it hard to dissolve (Sjöholm, 2003). The cellulose chains form in microfibrils, either in highly ordered (crystalline) regions or with less ordered regions, depends on the forming intra- and inter molecular hydrogen bonds (Krässig, 1993).

Hemicelluloses consist of polysaccharides with different monosaccharides as unit structure. Their structure contains shorter chains that are more branched as compared to cellulose. Hemicelluloses functions as a supporting material in the cell walls. The average degree of polymerisation of hemicelluloses is about 200 (Sjöström, 1993). The structure of hemicelluloses depends on the wood type (softwood or hardwood). Softwoods mainly consist of galactoglucomannans and arabinogluconuranoxylan, while hardwoods contain glucuronoxylan, but glucomannan also exists (Sjöström, 1993).

Lignin is one of the most abundant biopolymers in nature. It usually contributes between 20%-35% of wood dry matter depending on the different wood species (Glennie and Mc-Carthy, 1962). The role of lignin in plant structure is to act as a matrix material that binds the plant fibers (polysaccharide microfibrils) to impart enough strength to the plant stem for vertical growth (Feldman, 2002). Furthermore, lignin contributes to an efficient
nutrition and water transportation system by making hydrophobic cell wall (Ek et al., 2009). In trees, lignin creates a protective barrier to enzymatic attack around the cellulose (Stenius et al., 2000). In addition, lignin has important implications in agricultural soils for the soil organic matter cycling, thus affecting mineralization of nutrients, carbon sequestration and soil structure (Frei, 2013).
Table 1. Chemical composition of different wood and agricultural residues (% w/w), Percentage based on dry weight.

<table>
<thead>
<tr>
<th>Biomass type</th>
<th>Cellulose</th>
<th>Hemicelluloses/other polysaccharides</th>
<th>Lignin</th>
<th>Extractives</th>
<th>Ash</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softwood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picea glauca (White spruce)</td>
<td>39.5</td>
<td>30.6</td>
<td>27.5</td>
<td>2.1</td>
<td>0.3</td>
<td>(Sjöström, 1993)</td>
</tr>
<tr>
<td>Hardwood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eucalyptus camaldulensis (River red gum)</td>
<td>45.0</td>
<td>19.2</td>
<td>31.3</td>
<td>2.8</td>
<td>1.7</td>
<td>(Sjöström, 1993)</td>
</tr>
<tr>
<td>Agricultural residues</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice straw</td>
<td>39.2</td>
<td>23.5</td>
<td>36.1</td>
<td>-</td>
<td>12.4</td>
<td>(El-Tayeb et al., 2012)</td>
</tr>
<tr>
<td>Corn stalks</td>
<td>61.2</td>
<td>19.3</td>
<td>6.9</td>
<td>-</td>
<td>10.8</td>
<td>(El-Tayeb et al., 2012)</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>36-41</td>
<td>22-36</td>
<td>5-10</td>
<td>2.4-3.4 (protein)</td>
<td>5.5</td>
<td>(Shearman et al., 2005)</td>
</tr>
</tbody>
</table>
2.2 Lignin chemistry

Lignin is built up by coupling of three major phenylpropanoid units, namely sinapyl, coniferyl and p-coumaryl alcohols (Pettersen, 1984). The respective aromatic constituents of these alcohols in the polymer are called syringyl (S), guaiacyl (G) and p-hydroxyphenyl (H) moieties (Adler, 1977) (Figure 1).

![Structural units of lignin](image)

Figure 1. Structural units of lignin. Adapted from (Adler, 1977; Pettersen, 1984)

Lignins from various plants (softwoods, hardwoods and non-woods) are different in the percentage of each phenylpropanoid unit and the degree of carbon-carbon linkages between lignin units (Telmo and Lousada, 2011). On the other hand, lignins from the same source can be different in terms of their structures and functions depending on the conditions under which they are extracted (Bykov, 2008). Softwoods lignins are primarily comprised of guaiacyl with only small amounts of hydroxyphenyl and syringyl
units. Hardwood lignins are often referred to as guaiacyl-syringyl lignins, as they contain both types of lignins. Hardwoods also contain small amounts of hydroxyphenyl lignin (Holtman, 2003). Although, the ratio of phenylpropanoid units in non-wood lignins varies, they normally contains all three precursors, guaiacyl, syringyl and p-hydroxyphenyl (Derkacheva and Sukhov, 2008). The overall content of p-hydroxyphenyl is higher in annual crops than in softwoods and hardwoods (Brodin, 2009).

The monomers are linked by carbon-carbon or ether bonds polymerized by a radical coupling process. Many different linkage types may occur at any of several different locations on each phenolic unit. The most common linkage types in a lignin molecule are β-O-4, α-O-4, β-5, 5-5, 4-O-5, β-1, β-β and dibenzodioxocin (Figure 2) (Karhunen et al., 1995; Sjöström, 1993) which is about one-third of the linkages comprising of carbon-carbon and two-thirds are ether linkages (Sjöström, 1993).
The phenylpropanoid units bond to each other with a variety of carbon-carbon and ether bonds to make the lignin macromolecule (Figure 3) (Gösta and Knut, 2010). Types of linkages and dimeric structures of softwood and hardwood lignins revealed a high proportion of β-O-4 bonds (Table 2) (Derkacheva and Sukhov, 2008; Ek et al., 2009; Sjöström, 1993). The higher number of β-O-4 linkage in hardwood lignin resulted from radicals being limited to covalent bonds in the 5-position on the syringyl unit. The guaiacyl units in softwood create a more cross-linked and branched structure as
compared with that in hardwood lignins by making β-5 and 5-5 linkages in the free 5-position (Brodin, 2009).

Therefore, the molecular structure of lignin is expected to be very complex due to great variety of linkages and different types of functional groups (Figure 3) (Gösta and Knut, 2010). For these reasons, the exact molecular structure of randomized phenylpropanoid units of tri-dimensional network lignin was consequently difficult to predict (Sjöström, 1993). Although many models have been proposed for lignin in literature, no complete structure of a lignin has been identified due to its large complicated structure and the difficulties in lignin analysis. These models are only representations of each linkage and their lignin unit types.

Table 2. Different types of linkages between phenylpropanoid units in lignin as percent of total linkages (Sjöström, 1993)

<table>
<thead>
<tr>
<th>Linkage Type</th>
<th>Dimerstructure</th>
<th>% in Softwood</th>
<th>% in Hardwood</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-O-4</td>
<td>Arylglycerol-β-aryl-ether</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>α-O-4</td>
<td>Noncyclicbenzyl aryl ether</td>
<td>2-8</td>
<td>7</td>
</tr>
<tr>
<td>β-5</td>
<td>Phenylcoumaran</td>
<td>9-12</td>
<td>6</td>
</tr>
<tr>
<td>5-5</td>
<td>Biphenyl</td>
<td>10-11</td>
<td>5</td>
</tr>
<tr>
<td>4-O-5</td>
<td>Diarylether</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>β-1</td>
<td>1,2 –Diarylpropane</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>β-β</td>
<td>Linked through side chain</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
The structure of native lignin is changed during chemical or biological treatments. The main changes of lignin structure during pulping process depend on the isolation method used for delignification (Tejado et al., 2007). In the kraft process, β-O-4 and α-O-4 linkages are cleaved and produce a lot of non-etherified phenolic hydroxyl groups in lignin. In alkaline conditions, quinonemethide is formed by leaving the phenolic benzyl alcohol. The reaction reaches to equilibrium with connecting the hydrosulphide ion to the α-carbon on the lignin. An episulphide structure and a phenolic end group are formed when the hydrosulphide ion acts as a nucleophile on the β-carbon. Then, unstable episulphide generates elemental sulfur in the cooking liquor. Longer cooking process cleaves more β-O-4 linkages, and as a result, degraded lignin and free phenolic groups are increased (Norberg, 2012).

In the soda process of non-woody plants only small quantities of phenolic OH are produced after the cleavage of β-O-4 and α-O-4 linkages and some loss of primary aliphatic hydroxyls (Tejado et al., 2007).
2.2.1 Reactive functional groups in lignin

The major functional groups of lignin are aliphatic hydroxyl, phenolic hydroxyl, methoxyl, carbonyl, and uncondensed guaiacyl groups. It has been found that bulky side groups, such as methoxyl groups, expand intermolecular distances, while hydroxyl groups decrease their mean value (Janshekar and Fiechter, 1983).

The reactive functional groups on the three carbon atoms of the propane side-chain of the lignin are responsible for the typical reactions such as sulfonation by sulfite solution (Adler et al., 1957).

The phenolic hydroxyl groups (etherified or free) situated in the para position to the propane side-chain are favorable for many properties of technical and native lignins (e.g. solubility in alkalis) (Chudakov, 1961). In native lignin, only 10-13 % of the oxygen atoms in the carbon 4-position are free phenolic, while the rest of the oxygen in 4-position is linked to others units and form ether bonds (Ek et al., 2009). Phenolic structures require less redox-potential for being oxidized due to more possible resonance forms for the phenolic radicals than for nonphenolic. Therefore, the present of phenolic structure is a drawback for lignin biodegradation and many pulp bleaching methods which are based on oxidation of aromatic rings (Ek et al., 2009). On the other hand, the content of phenol in lignin is directly related to the reactivity of lignin in chemical pulping where the phenolic groups play a control role (Ek et al., 2009).

2.2.2 Lignin-Carbohydrate Complex (LCC)

Lignin is always associated with hemicelluloses (carbohydrates) in plant cell wall, not only as physical admixtures, but also through covalent bonds (Norberg, 2012; Sarkanen and Ludwig, 1971). The mixture builds a complex structure which is referred to as lignin-carbohydrate complex (LCC). The separation of lignin from carbohydrates cannot be accomplished by using conventional analytical methods such as ultracentrifugation, electrophoresis, hydrophobic-interaction chromatography and gel filtration (Janshekar and Fiechter, 1983).
The main lignin-carbohydrate linkages have been reported to be γ-esters, α-ether and phenyl glycosides (Balakshin et al., 2009). The linkages are formed between C-1 hydroxyl groups at the reducing-end of polysaccharides and the phenolic hydroxyl groups of lignin moieties (Fengel and Wegener, 1983). The lignin–carbohydrate complexes from herbaceous crops contain ferulic acids attached to lignin with ether bonds and to carbohydrates with ester bonds (Brandt et al., 2013; Himmelsbach, 1993; Lapierre and Monties, 1989) (Figure 4). Ester linkages between p-coumaric and ferulic acids and lignin have been confirmed in milled wood lignin of grasses by analytical and spectrophotometric procedures (Higuchi et al., 1967).

Figure 4. Lignin–carbohydrate complex in grass involving ferulic acid (Adapted from Brandt et al., 2013)
2.3 Delignification of lignocellulosic biomass

Many industrial processes exist for delignification from plant materials. These processes are producing plant fibers or hydrolyzing polysaccharides in pulping industries or biorefineries, while lignin is generally considered as a byproduct. Lignin is isolated from plant biomass through depolymerization and/or derivatization. Consequently, the chemical structure of lignin alters depends on these industrial isolation procedures. Therefore, since the chemistry of isolated lignin is changed, the polymeric properties of native lignin like glass transition temperature and molecular weight are difficult to estimate (Glasser et al., 2000).

The delignification processes are often performed through pulping process or biorefinery operations. There are different processes such as mechanical, steam explosion, kraft, soda, organosolv etc. Although lignosulfonates, kraft and soda lignins are produced in large quantities, other lignin types, such as organosolv, hydrolysis and ionic liquid lignins, are produced in rather smaller amounts which may evolve into industrial scale products (Gosselink et al., 2004b).

2.3.1 Wood pulping

In wood pulping processes, lignin is removed from wood fibers in order to use the fiber for paper production. Several methods exist for removing lignin, but the most common are kraft, sulfite and soda pulping.

2.3.1.1 Kraft process

The kraft process produces strong pulps for manufacturing liner boards, paper-bags and corrugated boards. In the kraft process, a solution of sodium hydroxide and sodium sulfide is utilized to treat wood chips at a temperature around 170 °C. The ether linkages of lignin molecules are cleaved through this treatment. Consequently, the number of phenolic hydroxyl groups of the lignin increases and the molecular weight
decreases. The phenolic groups are ionized in alkaline conditions, making lignin soluble.

In the kraft process, almost all lignin and a large part of the hemicelluloses are dissolved and turn into the “black liquor” (Theliander, 2007). The cooking conditions, such as temperature, time and alkalinity, depend on the source of wood and the target of pulp types. When pulping is carried on at harsh conditions, then more lignin is degraded and dissolved into the black liquor. After cooking, the pulp is processed by washing, bleaching and drying and is then converted into paper, board or tissue grades (Norberg, 2012).

The cooking liquors are regenerated again after evaporation of the black liquor, and the organic compounds in the black liquor are burnt in the recovery boiler to produce steam (Theliander, 2007). The dry content of black liquor mainly includes organic residue from pulping, and inorganic substances from cooking chemicals. The main organic compounds are lignin (39-54%), degraded carbohydrates (25-35%) and extractives (3-5%) (Sjöström, 1993). The inorganic portion is about 18-25% of the dry contents which are mainly Na$_2$S, Na$_2$SO$_4$, Na$_2$CO$_3$, Na$_2$S$_2$O$_3$, NaCl and NaOH (Li, 2011).

2.3.1.2 Sulfite pulping

The sulfite pulping is a chemical pulping process which uses a mixture of sulfurous acids to solubilize lignin through cleavage of lignin bonds. In 1900s, the sulfite pulping was the most important pulping process, but it was turn down by kraft pulping in the 1940s. Advantages of sulfite pulp are brighter, easier refined pulps and with less porous sheet than kraft pulps. Some disadvantages of sulfite pulp include weaker pulp than kraft, not easy process for all species of wood, long cooking cycles and fairly complicated chemical recovery (Biermann, 1996).

In sulfite pulping, sulfites are added to the wood chips for 4 to 14 hours at pH ranging from 1.5 to 5, depending on the counterion to sulfite. The sulfite process is usually carried out between 130 to 160 °C, depending on the chemicals used (Sjöström, 1993).
In this process, the sulfurous acid is quickly penetrated into the wood chip in the vapor form (SO₂). After the proper cooking time at the desired cooking temperature, the pressure is reduced from 90 to 40 psi. As a result, wood fibers are effectively separated by sudden decompression (Biermann, 1996).

The resonance – stabilized carbocations are the intermediates that are involved in the sulfite process. The intermediates are formed either by acidic cleavage of ether bonds or by protonation of carbon-carbon double bonds. However, most of the lignin degradation is carried out through acidic cleavage of ether bonds. The following reaction shows that the electrophilic carbocations (R⁺) react with bisulfite (HSO₃⁻) to give sulfonates (R-SO₃H) (Sjöström, 1993).

\[
R-\text{O-R'} + H^+ \rightarrow R^+ + R'\text{OH}
\]

\[
R^+ + HSO_3^- \rightarrow R-SO_3H
\]

The important variables in the sulfite process are base ion, wood species, maximum cooking temperature, liquor to wood ratio, cooking time and acid concentration. The strength of the sulfite pulp is medium with flexible and soft fibers. The sulfite pulps are easily bleached due to their low lignin content. Resinous species, such as Douglas-fir and southern pine, are not suitable to sulfite cooking, while spruce, hemlock and balsam fir are the preferred species (Biermann, 1996).

2.3.1.3 Soda process

The soda process was invented as the first chemical pulping method in 1845. In this process, lignocellulosic material is cooked in a pressurised reactor to 140-170°C in the presence of 13-16% sodium hydroxide (cooking liquor). The ratio of liquid to dry fiber is typically 5:1. In the soda process, lignin is dissolved and suspended in the liquid phase, and separated from the cellulose. The liquid phase, called black liquor is separated from the solid phase containing cellulose fibers, which is called pulp. Pulp is then processed to manufacture, paper and paper products, boards, composite materials and so on. The
soda process mostly applied to the pulping of non-woods, such as wheat straw, kenaf, hemp, bagasse and sisal (Doherty et al., 2011). Lignin is recovered from the black liquor for reuse in the process as fuel for the boilers (Doherty and Rainey, 2006).

2.3.2 The biorefinery

A biorefinery converts biomass-derived feedstocks and intermediates such as lignocellulosic material, algae, sugar and oil crops to products, such as biofuel, heat, energy and biobased chemicals (Norberg, 2012; Vishtal and Kraslawski, 2011a). Currently, most of the biorefineries focus only on the valorization of hemicelluloses and cellulose, while lignin is considered as low value residue (Doherty et al., 2011). It is important to note that in the future, lignin will be produced in large quantities in the biorefineries because the United State of America plans to replace 30% of fossil fuels with biofuels by 2030. This will generate more than 60 million tons of lignin by 2022 (Ragauskas et al., 2014) and about 225 million tons by 2030 (Sahoo et al., 2011b).

In biorefineries, there are various methods that can be used for producing fermentable carbohydrates. Each of these methods produces lignin byproduct with unique properties. The production of bioethanol from biomass typically involves a hydrolytic pretreatment, either autocatalyzed by biomass derived organic acid or catalyzed by added mineral acids, which occurs in autohydrolysis or steam explosion (Glasser and Wright, 1998; Ibrahim and Glasser, 1999; Lora and Glasser, 2002). These pretreatments make carbohydrates more susceptible to saccharification and fermentation, and also produce sulfur-free lignin (Lora and Glasser, 2002). The process then proceeds by either enzymatic treatment or continuing acid hydrolysis to extract depolymerized carbohydrates (Schell et al., 2004).

Lignin can be extracted from solid residues through different techniques (for instance, by using organic solvent or alkali solution), and recovered by the acid precipitation method. In addition, the lignin isolated from pretreatment contains less sugar and ash in comparison with other harsh methods such as kraft and soda (Glasser and Strickland, 1987; Glasser and Wright, 1998; Ibrahim and Glasser, 1999; Lora and Glasser, 2002).
2.4.1.1. Steam explosion process

In the steam explosion process (a pretreatment process for producing bio-ethanol), biomass is exposed to high steam pressure followed by quick pressure release, causing hydrolysis of hemicelluloses (and to a lesser extent on the cellulose) and cleavage of lignin-hemicelluloses bonds (Doherty et al., 2011; Li et al., 2007). In this process, the lignin structure breaks down and is depolymerized (Cara et al., 2006), and results in a decrease in β-O-4 structures (Li et al., 2007).

In steam explosion separation, less chemicals are utilized during the process and, as a result, the lignin undergoes less bond cleavage. Solubility of hemicelluloses in water and solubility of lignin in alkaline or organic solvents is increased, leaving the cellulose with a reduced degree of polymerization as a solid residue (Li et al., 2007). However, the lignin from the solid residue can be extracted by aqueous alkaline solutions (Fox, 2006).

2.4 Isolation methods for lignin recovery

Lignin-rich streams from pulp mills and biorefinery sites are considered as by-products which should be removed or recycled (Vishtal and Kraslawski, 2011b). There are two main techniques for lignin separation; filtration and precipitation. However, precipitation of lignin by lowering the pH is the most common method for the separation of kraft and soda lignins (Jönsson and Wallberg, 2009; Vishtal and Kraslawski, 2011b). It is difficult to separate soda lignin though filtration due to its highly hydrophilic structure (Doherty et al., 2011).

Generally, lignin from black liquor is obtained by precipitation with acid in two steps. The LignoBoost® process is used to recover lignin from kraft black liquor by acidifying it with carbon dioxide (Beis et al., 2010; Li, 2011). In the first step, carbon dioxide is used to reduce the pH of the liquor to 9-10 (Vishtal and Kraslawski, 2011b). In this step about 75% of the lignin is precipitated as a sodium salt. Lignin is obtained by suspending the salt in water and addition of sulfuric acid to pH below 3 (Li, 2011; Vishtal and
Kraslawski, 2011b). Finally, lignin is separated through a filtration process (Vishtal and Kraslawski, 2011b). The cost for the extraction of lignin by carbon dioxide precipitation is about $32- $50 per ton of lignin (Axelsson et al., 2006).

Abacherli and Doppenberg (2001) have patented the technique for separation of soda lignin without using CO₂. The lignin is precipitated from the black liquor solution by reducing the pH while at room temperature; subsequently, the mixture is heated to about 70 to 80°C to turn it from a gelatinous form into a filterable form. Lignin is separated by filtration, washed with water, and dried in an oven.

During cooking process, ether bonds cleavage, and lignin macromolecules are degraded gradually in the form of lignin sodium salt. Lignin in the black liquor is formed as a hydrophilic gel. Hydrogen ions are replaced with sodium ions when the black liquor is neutralized by acid. As a result, lignin is precipitated from the black liquor because lignin in this form is insoluble in water (Li, 2011).

\[2R-\text{ONa} + \text{H}_2\text{SO}_4 \rightarrow 2\text{ROH (Precipitate)} + \text{Na}_2\text{SO}_4\]

Hydrolysis lignin contains solid lignin residue and significant amounts of unhydrolyzed cellulose (Vishtal and Kraslawski, 2011b). The hydrolyzed lignin can be easily filtered through a fine mesh without using a sophisticated recovery method. However, recovered lignin contains various impurities (Vishtal and Kraslawski, 2011b). Hydrolyzed lignin was extracted by NaOH extraction for use in synthesis of polyurethane (Cheng et al., 2007).

2.5 Industrial lignins

Industrial lignin is significant because of its production as a by-product in the large scale pulping industries and biorefineries. The main objective of pulping and biorefinery is to separate the cellulosic part by removing lignin from the biomass. Large amounts of lignin extracted by the wood pulping industries and biorefineries annually are burned to produce energy for boilers (Lora and Glasser, 2002).
2.5.1 Kraft lignin

Kraft lignin is the most common form of industrial lignin produced at 42 million tonnes annually (about 85% of the lignin production worldwide) (Mai et al., 2000; Tejado et al., 2007). In modern pulp mills, extraction of lignin from the black liquor produces a surplus energy for the boilers and that can be utilized for the production of more valuable products (Jönsson and Wallberg, 2009). There are few commercial sources of kraft lignin in the market. Mead-Westvaco produces about 20,000 metric ton/year of softwood kraft lignin (Indulin) in South Carolina. In 2013, Domtar has started to produce Kraft lignin (Biochoice™) with about 75 ton/day from a plant in Plymouth, North Carolina. In addition, FPInnovations, has recently set up a successful pilot plant in Thunder Bay for extraction of lignin through LignoForce™ process with a capacity of 100 kg/day.

Kraft lignin contains specific features which are different from other industrial lignins. It contains high amount of phenolic hydroxyl group and some biphenyl and other condensed structures due to extensive cleavage of β-aryl bonds during severe cooking condition (Vishtal and Kraslawski, 2011a). Quinine, catechol and carboxyl groups are formed due to oxidative conditions during delignification process (Chakar and Ragauskas, 2004). The ash content of kraft lignin is up to 30%, which is reduced to around 1-5% by treatment and washing with diluted sulfuric acid (Mansouri and Salvadó, 2006; Vishtal and Kraslawski, 2011b). Information on the properties of kraft lignin is shown in Table 5.

2.5.2 Soda lignin

Soda lignin is obtained from soda-anthraquinone or soda pulping process which is a sulfur-free process (main difference with respect to the kraft lignin). Therefore, the chemical composition of soda lignin is closer to natural lignin than kraft lignin (Wörmeyer et al., 2011). Normally crops such as straws, flax, bagasse are used in soda process. ALM India is manufacturing high-purity non-wood lignins (Protobind) with a capacity of more than 10,000 metric tons/year.
Table 3 lists the properties of non-wood soda lignin from two different origins. The analytical data obtained with non-wood soda lignins showed that all soda lignins were common in low molecular weight, high phenolic hydroxyl content and relatively low (and variable) glass transition temperature (Lora and Glasser, 2002). However, some properties like thermal behavior of soda lignins depend on type (process and feedstock) and on the presence of contaminants (Lora and Glasser, 2002).

Table 3. A comparison between the properties of wheat straw and hemp soda lignins (Lora and Glasser, 2002)

<table>
<thead>
<tr>
<th>Property</th>
<th>Wheat straw</th>
<th>Hemp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OH/C9</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Phenolic OH/C9</td>
<td>0.8-0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Carboxyl/C9</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>Methoxyl/C9</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Tg (°C)</td>
<td>160-185</td>
<td>158</td>
</tr>
<tr>
<td>Mn (g/mol)</td>
<td>1800</td>
<td>-</td>
</tr>
<tr>
<td>Mw (g/mol)</td>
<td>3300</td>
<td>-</td>
</tr>
</tbody>
</table>

Soda lignin normally contains high contents of silica, as crops such as straws, flax, bagasse are used in soda process (Vishtal and Kraslawski, 2011b). Silica may co-precipitate with the lignin, reducing the quality of the product. Non-wood lignins contain more p-hydroxyphenyl units and carboxyl groups than wood lignins (Buranov and Mazza, 2008). More information on chemical properties of soda lignin can be found in Table 5.
2.5.3 Lignosulfonate (Sulfite lignin)

Lignosulfonate is isolated lignin from wood by the sulfite pulping. Softwood lignosulfonate and hardwood lignosulfonate are obtained from the concentrated waste pulping liquor by the Howard process after stripping and recovery of the sulfur (Lange et al., 2013). Lignosulfonate is produced about 1,000,000 tons/year originating from sulfite pulping (Gargulak and Lebo, 2000). The current annual production of Tembec Temiscaming is 90,000 metric tons (Gellerstedt et al., 2012).

The weight-average molecular weight (Mw) of majority lignin sulfonates can vary from 10,000 Da to 50,000 Da (up to 150,000 Da) (Vishtal and Kraslawski, 2011a). During cooking, sulfonate groups are bonded to benzylic carbon of the phenylpropane units in lignin with very high ranging from 0.4 to 0.6 per C9 unit (Fredheim et al., 2002). Ligninsulfonates are thus anionic polyelectrolytes, soluble in water, acid solutions and in high polar organic solvents (Lange et al., 2013). The properties of lignosulfonates are given in Table 5.

2.5.4 Organosolv Lignin

Organosolv lignin is obtained from the organosolv pulping process after separation of wood components by using organic solvents (Lange et al., 2013). Many types of organic solvents with different combinations of alkaline and acidic components have been proposed for enhancement of organosolv pulping process. The most common organosolv process is known as Allcel process which uses ethanol or a mixture of ethanol and water (Pye and Lora, 1991). Organosolv lignin can be isolated through precipitation with water followed by distillation to recover the solvent, or by solvent removal and recovery (El Hage et al., 2009).

Organosolv lignin is soluble in basic aqueous solutions and in many polar organic solvents, while it is not completely soluble in acidic aqueous solutions. The number-average molecular weight of organosolv lignins are normally less than 1000 Da (Lange et al., 2013) and the weight-average molecular weight is between 500 to 5000 Da (Vishtal and Kraslawski, 2011a). Chemical composition of organosolv lignin can be found in Table 5.
2.5.5 Lignin from Hydrolysis (Biomass conversion techniques)

Most biorefinery concepts offer advanced technology for dissolving the sugar (in wood) and used it for fermentation to produce ethanol, while the residue lignin is usually used as fuel (Hamelinck et al., 2005). Nakagame reported that the activity of enzymatic hydrolysis lignin is higher than kraft lignin which can be used in preparation of polymeric materials (Nakagame et al., 2011). Hydrolysis lignin contains high number of condensed structures with high molecular weight (Carrott et al., 2008). It is difficult to dewater the hydrolysis lignin due to its high sorption ability (Vishtal and Kraslawski, 2011b).

During the hydrolysis process, sulfur-free lignin is generated as a by-product along with the saccharification and/or fermentation of polysaccharides. Lignin is extracted from the pretreated biomass (for instance with aqueous alkali or with an organic solvent), and is recovered by acid precipitation. The level of contaminant such as sugar and ash is low. Table 4 shows properties of hardwood and straw lignins isolated from the steam explosion processes (Lora and Glasser, 2002).

Table 4. Typical properties of lignins isolated from steam explosion process (Lora and Glasser, 2002)

<table>
<thead>
<tr>
<th>Property</th>
<th>Hardwood</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OH/C9</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Phenolic OH/C9</td>
<td>9.6-9.8</td>
<td>8.5</td>
</tr>
<tr>
<td>Carboxyl/C9</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Methoxyl/C9</td>
<td>1.0-1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Tg (C)</td>
<td>113-139</td>
<td>125</td>
</tr>
<tr>
<td>Mn</td>
<td>900</td>
<td>400</td>
</tr>
<tr>
<td>Mw</td>
<td>2300-3000</td>
<td>1100</td>
</tr>
</tbody>
</table>
The chemical compositions of industrial lignins from different sources (i.e. softwood, hardwood, non-wood) were summarized by Vishtal and Kraslawski, 2011 (Table 5). Industrial lignins with diverse properties such as purity, molecular weight, functional groups and homogeneity have potential for a wide range of applications.

As it can be seen in Table 5, lignosulfonates and kraft lignin contain high ash and sulfur contents in comparison to other lignins. However, kraft lignin contains less ash and sulfur than lignosulfonate. High ash and sulfur content restricts utilization of lignins in some applications such as synthesis of polymers and low-molecular weight substances (Vishtal and Kraslawski, 2011b). In addition, lignins with lower sulfur content are more suitable for the value added applications include filler for polymers (Lora and Glasser, 2002; Sahoo et al., 2011b). It should be mentioned that sulfur is chemically reacted and bonded to lignin, therefore, it would be difficult to remove it from the lignin. Although non-wood soda lignins are almost sulfur–free, the ash and nitrogen content of these lignins are significantly high.

The percentage of nitrogen in each lignin varies due to the differences in source of the plant and additives (i.e. amino compounds for making the lignin compatible with different polymeric systems) in commercial lignins (Sahoo et al., 2011b).

Molecular weight varies from lignin to lignin. In certain applications low molecular weight is favorable, while, high molecular weight is more favorable for other applications. The molecular weight of organosolv lignins is the lowest among other industrial lignins, which make it soluble in certain solvents and facilitate its further processing such as filler in the inks, varnishes and paints (Belgacem et al., 2003). However, utilization of organosolv lignin in adhesives and binders is rather limited due to its low molecular weight (Vishtal and Kraslawski, 2011b). On the other hand, ultra-high molecular weight ligniosulfonates improves their plasticizing properties (Areskogh and Henriksson, 2011).
Table 5. Chemical composition of the industrial lignins (Vishtal and Kraslawski, 2011a)

<table>
<thead>
<tr>
<th>Chemical composition (%)</th>
<th>Kraft lignin</th>
<th>Soda Lignin</th>
<th>Lignosulfonate</th>
<th>Organosolv lignin</th>
<th>Hydrolysis lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>0.5-3.0</td>
<td>0.7-2.3</td>
<td>4.0-8.0</td>
<td>1.7</td>
<td>1.0-3.0</td>
</tr>
<tr>
<td>Moisture content</td>
<td>3.0-6.0</td>
<td>2.5-5.0</td>
<td>5.8</td>
<td>7.5</td>
<td>4.0-9.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>1.0-2.3</td>
<td>1.5-3.0</td>
<td>-</td>
<td>1-3</td>
<td>10.0-22.4</td>
</tr>
<tr>
<td>Acid soluble lignin</td>
<td>1-4.9</td>
<td>1.0-11</td>
<td>-</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.05</td>
<td>0.2-1.0</td>
<td>0.02</td>
<td>0-0.3</td>
<td>0.5-1.4</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.0-3.0</td>
<td>0</td>
<td>3.5-8.0</td>
<td>0</td>
<td>0-1.0</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>1,500-500</td>
<td>1,000-3,000</td>
<td>1,000-50,000</td>
<td>500-5,000</td>
<td>5,000-10,000</td>
</tr>
</tbody>
</table>
2.6 Current uses of lignin for value-added product

Over the past few decades, many researchers have tried to take advantage of lignin for many positive attributes. Some of the positive factors are: availability in large amounts, high energy content, compatibility with several basic chemicals, number of reactive points and a sustainable source of phenolic and aromatic compounds (Lindberg et al., 1989). In addition, researchers are also interested to take advantage of lignin as a renewable source to make products that could replace petroleum-based materials. However, moving lignin utilization from a laboratory to an industrial scale has been very limited due to the highly variable and complex structure of lignin (Lindberg et al., 1989).

Recently, research on lignin utilization has been developed based on three different strategies; utilizing lignin directly (without or with less modification), modification of lignin before utilizing and degradation of lignin to small molecules. The first strategy is based on the understanding of each of the industrial lignin properties and matching them to specific applications. The second method is to engineer the properties of the lignin by taking advantage of the numerous possible reaction sites on the lignin backbone by chemical modification. The last strategy is to degrade lignin to produce small chemical compounds that could be useful. Each of these methods has shown promising results at the laboratory scale, and few successful cases have been developed to an industrial scale (Fox, 2006).

Although, lignin is currently isolated from the industrial residues, the majority of isolated lignin is burned to produce energy for boilers and only a small portion is used for value-added products. So far, only few applications for lignin (about 1 to 2% of commercial lignin) have been realized by researchers on an industrial scale (Lora and Glasser, 2002). The limitation of lignin utilization for high value-added products is due to two major concerns; non-uniform structure and unique chemical reactivity of the technical lignin (Vishtal and Kraslawski, 2011b).

Sulfonated lignins (kraft and lignosulfonates) are mature products that have have been used as dispersant and binder for long time, however, the many new applications are
related to sulfur–free lignins (Lora and Glasser, 2002). Recently, non-sulfonated lignins have been suggested and demonstrated as feasible feedstocks for several uses in industrial practice. Non-sulfonated lignins are not only used in the same industrial applications in which sulfonated lignins are used (Fox, 2006), but also can be used for new applications where odor-release commonly observed with sulfonated lignins (Lora and Glasser, 2002) is a concern. These include materials for wood panel products, polyurethane foams, epoxy resins and automotive brakes (Lora and Glasser, 2002). However, non-sulfonated lignins have still suffered in part due to the fact that they are not a dependable and consistent industrial source. This situation has been improved through an increasing of ethanol production from agricultural residues (Fox, 2006). Beside bioethanol production, non-sulfonated lignins can be also obtained from two other sources; solvent pulping (organosolv) and soda pulping, particularly from agricultural residues and non-wood fiber crops (Lora and Glasser, 2002).

2.6.1 Kraft Lignin

Many researchers have evaluated the utilization of kraft lignin as an additive in thermoset systems, such as in phenol formaldehyde, polyurethane and epoxy thermosetting resins. The polyurethane systems studied contained 10 - 40% lignin that reacted with diisocyanates and poly(ethylene glycol) (Hatakeyama, 2002). Although these systems were able to produce high strength products, they were quite brittle. Recently, Mahmood et. al., (2013) reported very effective hydrolytic depolymerization of kraft lignin at a yield of 80-90% at 250-300ºC for 45-90 min. The aliphatic hydroxyl number of depolymerized lignin was found to be in the range of 236–352 mg KOH/g, which could be a potential biopolyol for the synthesis of polyurethane foam (Mahmood et al., 2013).

The phenolic structure of lignin represents a natural and sustainable source that replacing expensive and non-renewable phenols in the manufacturing of phenol-formaldehyde (PF) resins. Danielson and Simonson showed that up to 50% of the phenol in a commercial PF resin could be replaced by kraft lignin without any reduction in the panel properties. However, to achieve acceptable panel properties, the hot
pressing time was significantly increased due to the lower reactivity of lignin compared with phenol (Danielson and Simonson, 1998). Kraft lignin was also depolymerized using hydrolysis reaction to produce lower molecular weight of lignin for the synthesis of lignin-phenol-formaldehyde resoles (Siddiqui, 2013). The yield and relative molecular weight of depolymerized lignin at temperature of 300°C was relatively moderate around 71wt% and 1,200 g/mol, respectively. Depolymerized lignin with a lower molecular weight can be use for the synthesis of lignin-phenol-formaldehyde due to less steric hindrance, increased reactive sites and increased in content of phenolic hydroxyl per lignin unit (Siddiqui, 2013).

Kraft lignin could be useful in thermoplastic materials due to formation of well-suited aromatic structures as a free radical scavenger in commodity thermoplastics materials. Free radicals are formed in plastics by irradiation with ultraviolet light which causes polymer degradation. Gosselink and his colleagues found that kraft lignin could be a substituted for more expensive UV stabilizers in polyethylene with comparable or only minor effects on product performance (Gosselink et al., 2004a).

New thermoplastic was produced by using 85% kraft lignin due to the tendency of lignin to form intermolecular associations in solution at high concentrations. The mechanical properties, such as stiffness and strength of the thermoplastic, were comparable to that of many petroleum-based plastics (Li and Sarkanen, 2000).

Kraft lignin has also been examined as a precursor for the production of carbon fibers. Kadla et al. (Kadla et al., 2002) produced carbon fibers by spinning blends of hardwood kraft lignin with polyethylene, polypropylene, polyethylene oxide or polyethylene terephthalate. The yield of the carbon fiber in their process was greater using lignin precursors than petroleum precursors. The thermal properties and the miscibility of the polyethylene oxide blend were the most favorable carbon fiber properties.

In the review work on the incorporation of lignin into epoxy resins, one study found that the addition of 20% kraft lignin in a commercially available epoxy system the adhesive shear strength is doubled (Feldman, 2002).
2.6.2 Lignosulfonates

Lignosulfonates exhibit surfactant properties which can be used in several industries, such as water reducer in concrete, dispersant, additive in coal-water slurry and viscosity reducer (Lora, 2008). Lignosulfonates can be used without any further modification or purification, however some applications require some modification and purification to enhance their properties. Lignosulfonates are mostly used as additives to concrete because they reduce the required amount of water and improve the strength of the concrete. Lignosulfonates are also used as oil drilling muds to reduce the viscosity of the muds thus reducing the amount of energy for drilling. In addition, they can be used as binder in different applications such road dust control and animal feed pellets. Vanillin is a chemical which is derived from lignosulfonate lignin. A detailed list of many other special features of lignosulfonates is given by Lebo et al., [2000] and Glasser et al., [2000] (Glasser et al., 2000; Lebo et al., 2000).

2.6.3 Non-Sulfonated Industrial Lignins

Non-sulfonated lignins are phenolic polymers that can be used in many thermosetting formulations such as phenolic, isocyanate or epoxy (Glasser, 1989; Kelley et al., 1989). In addition to the cost advantage, some environmental advantages (such as reducing formaldehyde emission) can be also obtained by using lignin (Lora and Glasser, 2002).

Organosolv lignins can be also used as partial replacement for phenolic resin in the manufacturing of friction products. The use of 20% organosolv lignin in the phenolic resin formulation resulted 6.6% improvement in wear properties of brake pads (Nehez, 1998) produced by an automotive manufacturer in North America (Lora and Glasser, 2002). Recently, Cheng et al (2013) successfully produced bio-phenol-formaldehyde resins from organosolv lignin with a phenol substitution ratio up to 75-90%. Their results showed that the plywood sample glued by the bio-phenol-formaldehyde resin was comparable or stronger shear strength than those samples prepared with pure phenol-formaldehyde resin (Cheng et al., 2013).
Oriented strand board (OSB) panels exhibited same positive mechanical properties compared to the controls when they are pressed with 5 – 25% organosolv lignin in the phenol formaldehyde adhesive resin (Lora and Glasser, 2002). A study showed an improvement in bonding properties by replacing 50% of the phenol in a commercial PF resin with alkali extracted bagasse lignin (Khan et al., 2004a). Another study reported a 31% loading Novafiber (non-sulfonated lignin) into phenol formaldehyde resins without serious loss in the mechanical properties of adhesive strength (Gosselink et al., 2004a).

2.7 Advanced applications of lignin

In addition to the benefits of sustainability and the use of “green” raw materials, the polymers from natural resources offer great potential for the preparation of novel and advanced applications. This section discusses the new techniques that have been exploited to develop advanced materials from lignin with a controlled structure down to the micro or nano scale such as, lignin-based micro/nanoporous structures, nanotubes, nanofibers, micro/nanoparticles.

2.7.1 Lignin-based micro/nanoporous materials

Porous materials have very important potential in various applications such as catalyst, insulating material and adsorbent. One of the common methods for preparation of porous materials is sol-gel polymerization of phenolic resins (i.e. resorcinol-formaldehyde) (Pekala and Schaefer, 1993). Recently, several authors tried to use lignin as a potential source of phenol in this method.

Grishechko et al., (2013) used lignin in the phenol-formaldehyde formulation (Grishechko et al., 2013). Similarly, Chen et al., (2011) replaced 50% of resorcinol by lignin in the resorcinol-formaldehyde formulation (Chen et al., 2011). Lignin cannot completely replace the phenolics due to its high degree of substitution and, as a result, its low reactivity. The replacement of phenol by lignin causes a decrease in bulk density
due to the increase in the overall porosity. However, the total surface area is also reduced, thus limiting some properties such as insulating performances and adsorption capacity (Chen et al., 2011; Grishechko et al., 2013). The lignin-phenol-formaldehyde porous material can present an overall porosity more than 80% which is comparable in term of thermal conductivity with commercial insulating material, like polystyrene foam (Grishechko et al., 2013).

Forgacz et al., (2013) produced lignin-based porous material from black liquor using the High Internal Phase Emulsion (HIPE) (Forgacz et al., 2013a; Forgacz et al., 2013b). First the authors added surfactant and epichlorohydrin as crosslinker to the black liquor. Then, castor oil was added to the mixture and emulsified by an emulsification device. The emulsion was then heated to generate lignin crosslinking with epichlorohydrin. Finally, the castor oil was removed by using ethanol. Void sizes were obtained in the range from 5 μm to 25 μm based on nature and amount of the surfactant (Forgacz et al., 2013a; Forgacz et al., 2013b).

2.7.2 Lignin nanotubes

Lignin nanotubes can be used as smart delivery vehicles of DNA without the cytotoxicity associated with carbon nanotubes (Ten et al., 2014). Lignin nanotubes were synthesized based on cross-linking of lignin to alumina membrane followed by addition of peroxidase-mediated and dissolution of the membrane in phosphoric acid. Faria et al., (2012) reported on the use of lignin for the production of multi-walled carbon nanotubes for chemical sensor applications (Faria et al., 2012).

2.7.3 Lignin nanofibers

Lignin nanofibers were prepared by modifying hydroxyl groups of lignin films with Poly(N-isopropylacrylamide) (PNIPAM) through Atom transfer radical polymerization (ATRP) under aqueous conditions (Gao et al., 2012). Lignin nanofibers were also obtained by co-electrospinning of Alcell lignin solutions at room temperature without addition of any polymer (Lallave et al., 2007).
2.7.4 Synthesis of lignin beads

Lignin beads can be prepared by using emulsion-based techniques, when the lignin forms in the dispersed phase. Saidane et al., (2010) prepared lignin beads with size in range of 100-800 μm using this technique (Saidane et al., 2010). Lignin beads were prepared in an emulsion of highly concentrated lignin alkaline solution in 1,2-dichloroethane. Then, the lignin is crosslinked using epichlorhydrin inside the dispersed phase. The lignin beads were later functionalized to expose sulfonhydrazine groups on their surface. Finally, functionalized lignins were obtained with treatment of lignin by hydrazine hydrate and thionyl chloride. Functionalized lignins can be used in the wine industry for reducing carbonyl compounds responsible for sulfur dioxide binding in sweet wines (Blasi et al., 2007).

Chen and Liu (2011) prepared in a similar manner emulsion of black liquor suspension in a mixture of chlorobenzene and oil containing a surfactant (Chen and Liu, 2011). Lignin was polymerized by using a crosslikner (epichlorhydrin) inside the dispersed phase. The average size of the spherical lignin beads was 300-450 μm which could be used as adsorbent for amino acid L-lysine.

2.7.5 Lignin micro/nanocapsules

Formation of certain polymers into micro/nanocapsules under specific conditions is a very attractive process in many pharmaceutical applications (Freitas et al., 2005; Soppimath et al., 2001). Several recent publications reported the potential of lignin-based micro/nanocapsules able to encapsulate hydrophobic/hydrophilic drugs (Tortora et al., 2014; Yiamsawas et al., 2014).

Tortora et al., (2014) prepared the lignin microcapsules in an oil-in-water emulsion (Tortora et al., 2014). In this technique olive oil was added to a lignin aqueous solution and sonication was applied. Lignin is transferred to the oil-water interface due to its amphiphilic nature. Therefore, it is possible to “lock” the structure and obtain the lignin capsules. The particle sizes of the microcapsules were obtained in the range of 0.3-1 μm. The microcapsules were found to be stable in water suspension over a month.
period. Lignin microcapsules were found to be non-cytotoxic with great potential in biomedical and cosmetic applications (Tortora et al., 2014).

Yiamsawas et al., (2014) prepared lignin-based nanocapsules by emulsifying the lignin aqueous solution in an organic phase of cyclohexane containing toluene diisocyanate (TDI) and a surfactant (Yiamsawas et al., 2014). Hollow nanocapsules of lignin-based polyurethane were formed by polymerization of lignin polyurethane at the cyclohexane-water interface. The particle size and the wall thickness of the particles were found to be in the range of 162-220 nm and 10-20 nm, respectively. The long time stability of the capsules in both organic and aqueous phases was observed over several months.

Recently, Wurm and Weiss, (2014) developed a method to generate lignin hollow nanocapsules containing hydrophilic substances through polyaddition reaction of toluene diisocyanate (TDI) with lignin in an inverse mini-emulsion (Figure 5) (Wurm and Weiss, 2014).

![Synthetic of lignin nano-containers by inverse mini-emulsion (with permission from Yiamsawas et al., 2014)](image-url)
2.7.6 Lignin micro/nanoparticles

Preparation and characterization of lignin-based micro/nanoparticles has been recently reported by several authors. Asrar and Diang (2010) patented a method for production of lignin-based microparticles for controlled release of agricultural actives such as fertilizers, herbicides and pesticides. Lignin acetate microparticles were synthesized through a solvent evaporation method and loaded with imidacloprid. These kinds of formulations necessarily must be of a lower cost than, for example, pharmaceutical applications. It is important to provide such formulations that can be produced economically and efficiently. Moreover, because such formulations are usually applied directly to plants or into the soil, it is important that the particles are biodegradable, so that they do not persist in the environment (Asrar and Ding, 2010).

Lignin based hydroxymethyl and epoxy nanoparticles were prepared and characterized by Popa et al., (Popa et al., 2011). The results showed that the treatment of birch veneer with lignin nanoparticles complexes with copper resulted in a high stability of the timber products. Gonugunta et al., (2012) synthesized lignin nanoparticles by carbonization of a commercial lignin (Protobind 2400) with particle size in the range between 25 nm to 150 nm. (Gonugunta et al., 2012). Frangville et al., (2012) reported the formation of biodegradable lignin nanoparticles in ethylene glycol by gradually adding hydrochloric acid to the solution (Frangville et al., 2012). The particle size of nanoparticles obtained was less than 100 nm. The nanoparticles were found to be stable after crosslinking with glutaraldehyde and redispersion in water for up to a month in a wide range of pH from 1 to 9. Qian et al., (2014) prepared lignin nanosized colloidal spheres by gradual addition of non-solvent (water) to a solution of lignin acetate in tetrahydrofuran (THF) (Qian et al., 2014). The nanospheres with a hydrodynamic radius of about 100 nm were water dispersible and stable up to pH 12. However, acetyl groups were hydrolyzed above pH 12, and preventing colloidization due to electrostatic repulsion. Lignin nanoparticles were also prepared for use as filler in natural rubber composites (Jiang et al., 2013). In this method, lignin alkaline solution was added to cationic polyelectrolyte solution of poly(diallyldimethylammonium chloride). Lignin aggregates at high pH due to negative charges of deprotonated phenolic and carboxyl
groups. They could adsorbed the cationic polymers, resulting particles with hydrodynamic radius of about 200 nm.
CHAPTER 3 Characterization of lignins isolated from steam exploded residues and kraft black liquor

3.1 Introduction

Lignin is the second most abundant biopolymer on earth, after cellulose. Lignin is largely produced as a byproduct in biorefineries and pulping industries through various processes such as kraft, soda, organosolv, steam explosion and enzymatic hydrolysis. The pulp industry produces around 50 million tons of lignin per year (Mai et al., 2000), and bioethanol biorefinery refineries will produce an estimated 225 million tons of lignin by 2030 (Sahoo et al., 2011b). The majority of lignin produced today is burned as fuel in boilers, and only a small portion is used for the production of value added products (Doherty et al., 2011).

The physico-chemical properties of lignin depend on the type of plant material and the processing treatment (Bykov, 2008; Sahoo et al., 2011b). Molecular weight, elemental composition and number of functional groups have been shown to vary in various types of lignin (Cateto et al., 2008; Vishtal and Kraslawski, 2011b). These differences provide the opportunity to utilize isolated lignin in different value added applications. For instance, lignin has been utilized to synthesize phenol–formaldehyde resins where phenolic hydroxyl groups and free positions in the aromatic ring are the most critical characteristics (Abdelwahab and Nassar, 2011; Alonso et al., 2004; Khan et al., 2004b; Mankar et al., 2012; Sarkar and Adhikari, 2001a; Tejado et al., 2007; Zhang et al., 2013c).

Lignin is also used as a filler and reinforcing phase for polymer blends in polyethylenes, polypropylenes and polylactides, where the compatibility of lignin with the matrices is the critical factor (Sahoo et al., 2011a). For instance, presence of polar groups in lignin makes it more compatible with polar polymers like polyesters or polyvinylchloride compared to polyolefins (non-polar polymers) (Cazacu et al., 2004; Gosselink et al., 2004c; Hatakeyama et al., 2005; Kadla et al., 2002; Lora and Glasser, 2002; Reza
Barzegari et al., 2012; Schorr et al., 2014). The compatibility of lignin with polyolefins could be improved through some modifications such as esterification (Schorr et al., 2014). Lignin has also been used as polyol (either direct utilization or after chemical modification) for production of certain polymers such as polyurethane (Cateto et al., 2008; Huang and Zhang, 2002; Mahmood et al., 2013; Sarkar and Adhikari, 2001b).

This work summarizes the physico-chemical characteristics of lignin isolated from various industrial sources (i.e. pulping and biorefinery) and their potential value added applications. Industrial lignins with different properties such as molecular weight, functional groups and elemental compositions have potential for a wide range of industrial applications.

### 3.2 Experimental

#### 3.2.1 Lignin samples and lignin isolation processes

L1 and L2 were isolated from the bioethanol biorefinery residue (L1-Orig.) and kraft black liquor (L2-Orig.), respectively. Three commercial lignins were also used in this thesis for comparison; L3 (Indulin AT, softwood kraft pine lignin) supplied by Westvaco Co., L4 (Protobind 2000) and L5 (Protobind 1000) non-wood (agricultural fibrous feedstock such as wheat straw) soda lignins supplied by ALM Private Limited. L3-I and L5-I were isolated from the commercial lignins L3 and L5, respectively. The raw materials and lignin samples are presented in Table 6.

L1 was isolated from the remaining part of a mixture of hardwood and non-wood species after steam explosion pretreatment, using isolation method described by Abacherli and Doppenberg 2001. Solid residue from bioethanol biorefinery production was ground and sieved to pass a 40 mesh size sieve. 20g of the fine powder was stirred in 100 mL caustic solution (0.5M) for 30 minutes. The solution was filtered with a Buchner funnel with 1 µm pore filter paper. The filtrate was precipitated by reducing the pH to 2 by adding H$_2$SO$_4$ (1M) and subsequently heated to 70°C. The resulting
precipitate was separated by filtration, washed with water at 50 to 60°C to remove degraded sugars and unreacted compounds, and then dried at 50°C overnight.

L2 was isolated from black liquor followed the method described by Tejado et al., 2007 (Tejado et al., 2007). The black liquor was produced in a kraft pulp mill in Brazil using eucalypt species. The initial pH of the black liquor was 12.9. After lowering the pH to about 2 by adding a solution of H₂SO₄ (1M), the precipitated lignin was filtered on a Buchner funnel and washed with water at 50 to 60°C, and then dried at 50°C overnight.

L3-I and L5-I were isolated through acid precipitation from two commercial lignins (L3 and L5) for a comparison with other isolated lignins. The isolation processes is illustrated in Figure 6.
Table 6. Original source of lignin samples

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Lignin isolation Treatment</th>
<th>Raw material</th>
<th>Origin</th>
<th>Main Species</th>
<th>Industry</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-Orig</td>
<td>As received</td>
<td>Bioethanol biorefinery residue</td>
<td>Hardwood/ Non-wood</td>
<td>Unknown</td>
<td>Bio-Ethanol</td>
<td>Steam Explosion/ Enzymatic Hydrolysis</td>
</tr>
<tr>
<td>L2-Orig</td>
<td>As received</td>
<td>Kraft black liquor</td>
<td>Hardwood</td>
<td>Eucalyptus</td>
<td>Pulp</td>
<td>Kraft</td>
</tr>
<tr>
<td>L1</td>
<td>Isolated (Abacherli and Doppenberg, 2001)</td>
<td>Bioethanol biorefinery residue</td>
<td>Hardwood/ Non-wood</td>
<td>Unknown</td>
<td>Bio-Ethanol</td>
<td>Steam Explosion/ Enzymatic Hydrolysis</td>
</tr>
<tr>
<td>L2</td>
<td>Isolated (Tejado et al., 2007)</td>
<td>Kraft black liquor</td>
<td>Hardwood</td>
<td>Eucalyptus</td>
<td>Pulp</td>
<td>Kraft</td>
</tr>
<tr>
<td>L3</td>
<td>As received</td>
<td>Commercial lignin (Indulin AT)</td>
<td>Softwood</td>
<td>Pine</td>
<td>Pulp</td>
<td>Kraft</td>
</tr>
<tr>
<td>L4</td>
<td>As received</td>
<td>Commercial lignin (Protobind 2000)</td>
<td>Non-wood</td>
<td>Wheat straw</td>
<td>Pulp</td>
<td>Soda</td>
</tr>
<tr>
<td>L5</td>
<td>As received</td>
<td>Commercial lignin (Protobind 1000)</td>
<td>Non-wood</td>
<td>Wheat straw</td>
<td>Pulp</td>
<td>Soda</td>
</tr>
<tr>
<td>L3-I</td>
<td>Isolated (Tejado et al., 2007)</td>
<td>Commercial lignin (Indulin AT)</td>
<td>Softwood</td>
<td>Pine</td>
<td>Pulp</td>
<td>Kraft</td>
</tr>
<tr>
<td>L5-I</td>
<td>Isolated (Tejado et al., 2007)</td>
<td>Commercial lignin (Protobind 1000)</td>
<td>Non-wood</td>
<td>Wheat straw</td>
<td>Pulp</td>
<td>Soda</td>
</tr>
</tbody>
</table>
3.2.2 Characterization methods

3.2.3.1. Determination of bulk density

The bulk density of the air-dried lignin samples was determined using the ASTM (C29/C29) standard method.

3.2.3.2. Determination of moisture content

The moisture content of lignin samples was determined using TAPPI T264-cm97. This moisture corresponds to the equilibrium of the moisture content of lignin samples in open lid container. This value will be taken into account for subsequent analysis.
3.2.3.3. Yield of extracted lignin from industrial residue

The yield of extracted lignin was determined as the weight ratio of the oven-dried isolated lignin to the oven-dried crude lignin:

\[
\text{Yield of extracted lignin (\%)} = \left( \frac{\text{mass of isolated lignin}}{\text{mass of crude material}} \right) \times 100
\]  

(1)

3.2.3.4. Ash content

The ash content of the lignin samples was gravimetrically determined in a muffle furnace at 525 °C (TAPPI T211). About 0.5 g of oven-dried lignin sample was weighed into tared ceramic dishes and put in a muffle furnace at 525±25 °C for 4 h. The samples turned white/gray at the end of the heating cycle. Samples were reweighed and the ash content was determined as follows:

\[
\text{Ash Content (\%)} = \left( \frac{\text{mass of ash}}{\text{mass of oven dried sample}} \right) \times 100
\]  

(2)

3.2.3.5. Chemical characterization of lignins

Klason lignin is defined as the solid residual material when a sample is subjected to hydrolysis treatment with 72% sulfuric acid. Klason lignin was determined according to TAPPI T222 standard method. Lignin samples were treated with 72% H₂SO₄ for 1 h in a water bath (30ºC), then diluted to 4% and autoclaved at 121ºC for 1 h. The hydrolyzed solution was vacuum filtered on a gooch filtering crucible (medium pore size) and dried in oven at 105ºC for 3 h. The Klason lignin was calculated as a percentage of the weight of the dry lignin sample.

Acid-soluble lignin was measured using UV spectroscopy (Maekawa et al., 1989; Zhu et al., 2013). The filtrate collected from the Klason lignin procedure, was neutralized with
calcium carbonate and filtered through 0.2 µm syringe filters. Acid-soluble lignin was
determined from the absorbance at 205 nm, according to the following equation:

\[
\text{Acid soluble lignin (\%) } = \frac{d \times V \times A_a}{a \times W \times L} \times 100
\]  \hspace{1cm} (3)

Where;

d is the dilution ratio (dimensionless)

V is the filtrate volume (L)

A_a is the absolute absorbance of the sample (dimensionless)

a is absorptivity of the lignin (L/g.cm)

W is the oven-dry mass of the sample (g)

L is path length of UV-Vis cell (cm)

The value of “a” was 110 at 205 nm.

3.2.3.6. Elemental analysis

The relative proportions of carbon, hydrogen and nitrogen in the air-dried samples was
determined with a Perkin Elmer Model 2400II CHN analyzer. Calibration was done with
acetanilide before each test. The air-dried sample weight was corrected for moisture.
The total sulfur content of the oven-dried lignin samples were evaluated by the
microwave acid digestion, and inductively coupled plasma atomic emission
spectroscopy (ICP-AES) analysis. The percentage of oxygen was obtained by
subtracting the sum of C, H, N and S contents from 100 percent (including the ash)
(Schorr et al., 2014). However, the nitrogen and sulfur could be included in ash content
as well. We assumed that nitrogen and sulfur are totally bonded to lignin. Svensson
(2008) reported that approximately 70% of the sulfur content in the softwood kraft lignin
is organically bound sulfur, while the rest of the sulfur content is inorganic (~29%) and elemental sulfur (~1%) (Svensson, 2008).

\[ C(\%) + H(\%) + N(\%) + S(\%) + O(\%) + ASH(\%) = 100 \]

or

\[ O(\%) = 100 - C(\%) + H(\%) + N(\%) + S(\%) + ASH(\%) \]

Empirical formula of lignin samples was obtained from the cumulative analysis of all elements.

\[ C(\%) + H(\%) + N(\%) + S(\%) + O(\%) = 100 \]

3.2.3.7. Determination of number of carboxyl groups by titration

The carboxyl groups were determined by titration of lignin in ethanol with sodium hydroxide (Gosselink et al., 2004b). First, sufficient volume of 0.1 M sodium hydroxide solution was added to 100 ml 95% (v/v) ethanol in water to adjust the pH to 9.0. Then 1.0 g of oven-dried lignin was added to the mixture and stirred for 10-15 min. Subsequently the mixture was titrated back to pH 9.0 with 0.1 M sodium hydroxide solution. The number of carboxyl groups (mmol) was calculated per 1.0 g of lignin.

3.2.3.8. Determination of total hydroxyl number by titration

The methodology for determination of hydroxyl content followed the ASTM D-4274-11. This method was developed for the determination of hydroxyl number of polyester and polyether polyols. Briefly, the blank and the oven-dried lignin samples were refluxed at 98 °C in 20 mL of an acetylation reagent solution. This solution was prepared by the mixture of 12.7 mL of acetic anhydride with 100 mL of dry pyridine. After refluxing for 2 h, the flasks were allowed to cool at room temperature. Then the excess of acetic anhydride was hydrolyzed with 30 mL of distilled water, and subsequently titrated with sodium hydroxide (0.5M). The total hydroxyl content was calculated from the difference
between the acetic acid concentration of the blank and that of the lignin samples. The amount of sample was adjusted in such a way that required the volume of 0.5 M sodium hydroxide solution used for the titration of the lignin sample to be less than 80% of that required for the blank. The hydroxyl content in (mmol/g) sample was calculated by the following equation;

\[
Hydroxyl \ content \ (\text{mmol/g}) = [(B - A) \times N/W] - C \tag{4}
\]

where,

A is the volume (ml) of sodium hydroxide solution for titration of the acetylated lignin solution

B is the volume (ml) of sodium hydroxide solution for titration of the blank solution

N is the normality of the sodium hydroxide solution

W is the weight (g) of the sample

C is the number of carboxyl groups (mmol/g)

3.2.3.9. FTIR Analysis

FTIR analysis was carried out by using a FTIR Varian 600-IR, equipped with a Mercury Cadmium Telluride (MCT) detector and attached with ATR unit (PIKE MIRacle). Air-dried lignin samples (in powder form) were put into the sample compartment of the ATR and pressed against the diamond crystal. Similar pressure was applied for all measurement by using the pressure applicator attached with a torque knob. The wave number range was chosen in the 4000-600 cm\(^{-1}\) range with a 150 scan and resolution of 4 cm\(^{-1}\). The spectra were detected in absorption mode. Background scanning and correction were performed before running new sample. The relative peak absorbance was normalized (the intensity of highest absorbance peak normalized to unity) for all the IR bands of each lignin sample.
3.2.3.10. $^1$H-NMR Spectroscopy

The $^1$H-NMR of acetylated lignins was carried out by a Varian Unity Plus 500 MHz spectrometer following the method described by (Li and Lundquist, 1994). The experiment was operated in the quadrature mode. Typical $^1$H-NMR data points were recorded by accordance with the acquisition time of 4.0 s, number of scans of 126 and relaxation time of 1.0 s at room temperature. The lignin was acetylated by a 1:1 pyridine and acetic anhydride solution at 90°C for 3 h in a sealed flask.

3.2.3.11. $^{31}$PNMR Spectroscopy

Total aliphatic hydroxyl, phenolic hydroxyl and carboxyl groups, and G/S/H ratio of lignin samples were determined by quantitative $^{31}$P NMR using published procedures (Cateto et al., 2008; Granata and Argyropoulos, 1995). A proper solvent solution of pyridine and CDCl$_3$ (1.6/1, v/v) was prepared for dissolving lignin and other reagents. Phosphitylation of lignin samples was performed with using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as a phosphitylating reagent (Figure 7) (Yáñez-S et al., 2014). The internal standard solution (cholesterol, 85 mg/mL) and the relaxation reagent solution (chromium(III) acetylacetonate, 5.6 mg/mL) were prepared with the same solvent solution. 40.0 mg of oven-dried lignin was dissolved in 500 µL of the solvent solution in a sealed vial; this was followed by the addition of 100 µL of the internal standard and 50 µL of relaxation solution. Then, 100 µL of the phosphitylation reagent was added, and the vial was shaken to ensure a homogeneous mixture. After derivatization, the resulting solution was transferred to a 3-mm tube, and the $^{31}$P-NMR spectrum was recorded by a Varian Unity Plus 600 MHz spectrometer.
Figure 7. The reaction of lignin with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) for quantitative $^{31}$P NMR analysis (Adopted from Yáñez-S et al., 2014)

3.2.3.12 Solubility of lignin in alkaline solution

A Varian Cary 5000 UV-Vis NIR spectrophotometer (Agilent Technologies, CA, USA) was employed to determine the solubility of lignin in alkaline solution. 1.0 g oven-dried lignin sample was dissolved in 30 mL NaOH with a series of concentrations ranging from 0 mol/L to 0.5 mol/L. The solutions were shaken for overnight, and soluble part was separated by using a centrifuge at 9000rpm for 15 min. Then, 0.5mL of the supernatant was collected and diluted to an appropriate concentration to detect with UV Spectrophotometer.

3.2.3.13 High Performance Size Exclusion Chromatography (HPSEC)

Molecular mass distributions of four lignin samples were analyzed by the method described by Gonzalez 2000 (González et al., 2000). In this method High Performance Size Exclusion Chromatography (HPSEC) was performed to determine the molecular mass distribution of lignin sample in alkaline solution. HPSEC of different lignins was
carried out with a DIONEX DX600 chromatograph equipped with an UV detector and a PSS MCX column (1000 Å, 300 × 8 mm). The UV detection was carried out at the wavelength of 280 nm at room temperature (25 °C). UV detector was adjusted at 280 nm due to the maximum UV absorption of the lignins. This wavelength was used to estimate for molar concentration of the aromatic rings. The injection was 25 μL. Eluent (0.1 M NaOH solution) was prepared with deionized water (Millipore water from a purification system). Sodium poly(styrene sulfonate) which is known to exhibit a similar behavior with lignin was used for calibration of the column. Sodium poly(styrene sulfonates) standards (6520, 4230, 1830 and 1100 daltons) were purchased from Polymer Standard Services - USA Incorporation. Calibration curve was prepared by adding 10 mg of each polystyrene standard in 10 mL water. Each lignin sample was prepared by dissolving 10 mg of the dry lignin in 100 mL of 0.1 M sodium hydroxide solution. The stationary phase of this column is sulfonated styrene-divinylbenzene copolymer-network which is appropriate for carrying out HPSEC experiments over the whole 7-13 pH range. The number and weight average molecular weights were calculated based on the ASTM D5296 –11.

3.2 Results and discussion

3.2.3 The yield percentage of isolated Lignin

The amount of lignin isolated from the bioethanol biorefinery residues was about 38% on dry weight of lignocellulosic residue and about 27% of black liquor solids. Yield of lignin from the residues is of course an important consideration, as it will affect the economics of the recovery process. The percentage of extracted lignin from the black liquor falls within the expected range of 20 to 40% lignin based on the dry content (Vishtal and Kraslawski, 2011a).

3.2.4 Optical microscopic images of isolated lignins and their origins

The optical microscopic images showed obvious differences between the isolated lignins and their origins (Figure 8). A comparison between the residues from bioethanol biorefinery and pulping industry show that the steam explosion process generates more fibers than kraft process.
<table>
<thead>
<tr>
<th>Isolated lignin</th>
<th>Original source of the lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image 1](118x572 to 296x705)</td>
<td>![Image 2](317x572 to 495x705)</td>
</tr>
<tr>
<td><strong>L1</strong> - Isolated lignin from biorefinery residue</td>
<td><strong>L1-Orig.</strong> Bioethanol Biorefinery residue</td>
</tr>
<tr>
<td>![Image 3](118x426 to 296x560)</td>
<td>![Image 4](317x426 to 495x560)</td>
</tr>
<tr>
<td><strong>L2</strong> - Isolated lignin from black liquor</td>
<td><strong>L2-Orig.</strong> Dried black liquor</td>
</tr>
<tr>
<td>![Image 5](120x281 to 296x414)</td>
<td>![Image 6](317x281 to 495x414)</td>
</tr>
<tr>
<td><strong>L3</strong> - Commercial lignin</td>
<td><strong>L3</strong> - Commercial lignin</td>
</tr>
<tr>
<td>![Image 7](119x135 to 296x268)</td>
<td>![Image 8](317x135 to 495x268)</td>
</tr>
<tr>
<td><strong>L4</strong> - Commercial lignin</td>
<td><strong>L4</strong> - Commercial lignin</td>
</tr>
</tbody>
</table>

Figure 8. Microscopic images of isolated lignins and their original source. The scale bar is 0.5mm (NA; not available)
3.2.5 Analysis of lignin

The composition of the lignin samples can be seen in Table 7. There are some noticeable differences among the samples, with the industrial lignins (L3 & L4) possessing higher amounts of Klason (or acid insoluble) lignin and lower amounts of acid-soluble lignin than the lignins isolated from the industrial residues (L1 & L2). The percentage of acid-soluble lignin was calculated based on the absorbance at 205nm and Eq. 3 (Figure 9). Schorr et al. 2014, reported the Klason’s lignin content for Indulin AT to be 93%, similar to L3 sample.

The amount of inorganics (ash) are quite low (< 2%) for all samples with the exception of the L3, the commercial softwood kraft lignin which contains more than 4%. A similar ash content of 3.59% was reported by Schorr et al 2014. L1 has the lowest ash content of the four samples. Unlike the L2, L3, and L4 derived as residue from chemical pulping processes, L1 is derived from residue from a bioethanol process. In the bioethanol process a steam explosion pretreatment followed by enzymatic hydrolysis and fermentation is employed and hence there is no add of chemicals/salts to the process. Hence the low ash in L1 may be favorable for certain applications as ash is considered a contaminant and depending on which elements are present, could cause negative effects during further processing.

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble Klason lignin</td>
<td>85.1±3.9</td>
<td>86.3±0.9</td>
<td>91.0±0.45</td>
<td>91.2±0.4</td>
</tr>
<tr>
<td>Acid-soluble lignin</td>
<td>5.4±2.0</td>
<td>6.0±1.8</td>
<td>2.1±1.0</td>
<td>3.9±1.1</td>
</tr>
<tr>
<td>Ash</td>
<td>0.45±0.09</td>
<td>1.54±0.13</td>
<td>4.25±0.08</td>
<td>1.38±0.11</td>
</tr>
<tr>
<td>Total</td>
<td>90.9</td>
<td>93.8</td>
<td>97.4</td>
<td>96.5</td>
</tr>
</tbody>
</table>
3.2.6 Bulk Density of lignin samples

The bulk density of lignin samples isolated from bioethanol biorefinery (L1) was 380 kg/m³ and that for kraft lignin (L2) was 420 kg/m³. The bulk density for two commercial lignins was 460 kg/m³ (L3) and 550 kg/m³ (L4). The bulk density of soda lignin was reported 680 kg/m³, which was higher than the values reported for soda lignins (450-500 kg/m³) (Mousavioun and Doherty, 2010).

3.2.7 FTIR Spectroscopy

FTIR spectra of the two raw materials (bioethanol biorefinery residue and black liquor) and their isolated lignins (L1 and L2) are exhibited in Figure 10. Significant differences were observed between the spectra of two raw materials and their isolated lignins.

In the L1 spectrum, a considerable decrease was observed in the absorption intensities at the bands 1158 cm⁻¹, 1055 cm⁻¹ and 1030 cm⁻¹ after isolation from bioethanol biorefinery residue. The absorption bands in the bioethanol biorefinery residue at 1158 cm⁻¹ show a C–O–C asymmetric vibration and 1055 cm⁻¹ and 1030 cm⁻¹ indicate C–O stretch of cellulose (glycosidic linkages) (Adsul et al., 2011; Corredor et al., 2009). In the steam explosion process glycosidic bonds in the hemicelluloses (and to a lesser extent
in the cellulose) are hydrolyzed. Hemicellulose–lignin bonds are also cleaved in this process. Solubility of hemicelluloses in water and solubility of lignin in alkaline or organic solvents is increased, leaving the cellulose with a reduced degree of polymerization (Li et al., 2007). Therefore, the appearance of these strong bands could be attributed to cellulose and hemicelluloses contaminants in the bioethanol biorefinery residue sample (She et al., 2010).

Differences in the kraft black liquor and L2 spectra were mostly observed in the area of aromatic ring vibrations (e.g. bands at 1577, 1492, 1445 and 1414 cm\(^{-1}\)). The intensity of those bands was stronger than the intensity of the same absorption bands in the spectrum of isolated L2. This is due to the presence of low molecular weight lignin-like compounds which are created in the kraft process, but then removed during isolation process (Gellerstedt and Lindfors, 1984).

![Graph](image)

Figure 10. Comparison between the raw materials and their isolated lignin; L1 (isolated from bioethanol biorefinery residue) and L2 (isolated from kraft black liquor)

Figure 11a shows a comparison between the spectra of four lignin samples. Only minor differences were observed between the samples due to similar functionalities but they differ from one another by the percentages of functional groups. The absorption bands were strongly consistent with assigned band of chemical components as mentioned in
previous literature (Anglès et al., 2003; Derkacheva and Sukhov, 2008; Zhou et al., 2011). The information regarding corresponding bands is given in Table 8. A wide band was observed for all isolated lignins in the 3500-3100 cm\(^{-1}\) wavenumber range. This band typically refers to the presence of hydroxyl groups (alcoholic and phenolic) involved in hydrogen bonds. The two bands at 2900 cm\(^{-1}\) and 2800 cm\(^{-1}\) correspond to methyl (−CH\(_3\)) and methylene (−CH\(_2\)) groups. The position of the bands at 1705 cm\(^{-1}\) and 1595 cm\(^{-1}\) can be attributed to non-conjugated and conjugated carbonyl groups which were observed in all lignin spectra. The carboxyl groups in lignin samples are represented between 1750 and 1550 cm\(^{-1}\) (Gosselink et al., 2004b). Absorption bands at 1595 cm\(^{-1}\) and 1513 cm\(^{-1}\) were assigned to aromatic skeletal vibration of lignins (Camargo et al., 2012; Zhou et al., 2011). In the 1460-1420 cm\(^{-1}\) wavenumber range, two intense bands were observed. These bands corresponded to C−H stretching band (methyl and methylene) and C−H in-plane deformation with aromatic ring stretching (Awal and Sain, 2011; She et al., 2010; Zhou et al., 2011).

In order to discern the differences in lignin structure, Figure 11b exhibits the region of 1400-1000 cm\(^{-1}\). The phenolic OH groups in lignin (band at 1365 cm\(^{-1}\)), are produced during chemical process when β-O-4 linkages are cleaved and generate non-etherified hydroxyls. Low intensity of this band in L1 spectrum shows that small portion of phenolic OH group is generated (due to less β-O-4 linkage cleavage) during steam explosion process. The stretching vibration of C-O bond in syringyl rings can be observed at 1325 cm\(^{-1}\). This band did not appear in the L3 spectrum due to absence of syringyl units in softwood lignin (Anglès et al., 2003; Awal and Sain, 2011).

Furthermore, it can be observed that the intensity of absorption bands at 1266 cm\(^{-1}\) (C-O stretching of guaiacyl ring) in L3 spectra is stronger than other lignins because guaiacyl is dominant lignin unit in softwoods. The intensity of absorption bands at 1213 cm\(^{-1}\) and 1150 cm\(^{-1}\) were observed with equal intensity for all spectra, which probably referred to in-plane deformation vibration of both guaiacyl C−H and syringyl C−H (Pandey, 1999). On the other hand, the intensity of the band at 1112 cm\(^{-1}\) (C-H deformation in syringyl ring) appeared with higher intensity for L2 compared to L4 which suggests a higher percentages of syringyl in the hardwood sample than the non-wood.
sample. The absorption band range 1030-1025 cm$^{-1}$ is assigned to deformation vibration of C–H bonds in the guaiacyl ring and also assigned to C-O bonds in both syringyl and guaiacyl. This absorption band appears in higher intensity in L3 compared to others due to higher content of guaiacyl type lignin in softwoods.

Absorption bands at 851 cm$^{-1}$ and 812 cm$^{-1}$ refer to the deformation vibration of C–H bonds in the aromatic ring of guaiacyl ring in L3 (Figure 11a). However, the absorption bands for vibration of C–H bonds of syringyl ring were observed in 830-820 cm$^{-1}$ for L1, L2 and L4 (Mansouri et al., 2011).

Figure 11. FTIR Spectra of isolated lignin samples in the range of (a) 4000-600cm$^{-1}$ and (b) 1400–1000 cm$^{-1}$
Table 8. Fourier transform infrared of four lignin samples

<table>
<thead>
<tr>
<th>Signal No.</th>
<th>Band position (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L1</td>
<td>L2</td>
</tr>
<tr>
<td>1</td>
<td>3500-</td>
<td>3100</td>
</tr>
<tr>
<td>2</td>
<td>2939</td>
<td>2939</td>
</tr>
<tr>
<td>3</td>
<td>2833</td>
<td>2833</td>
</tr>
<tr>
<td>4</td>
<td>1705</td>
<td>1705</td>
</tr>
<tr>
<td>5</td>
<td>1595</td>
<td>1595</td>
</tr>
<tr>
<td>6</td>
<td>1513</td>
<td>1513</td>
</tr>
<tr>
<td>7</td>
<td>1458</td>
<td>1458</td>
</tr>
<tr>
<td>8</td>
<td>1426</td>
<td>1426</td>
</tr>
<tr>
<td>9</td>
<td>1365</td>
<td>1365</td>
</tr>
<tr>
<td>10</td>
<td>1326</td>
<td>1326</td>
</tr>
<tr>
<td>11</td>
<td>1268</td>
<td>1268</td>
</tr>
<tr>
<td>12</td>
<td>1213</td>
<td>1213</td>
</tr>
<tr>
<td>13</td>
<td>1151</td>
<td>1151</td>
</tr>
<tr>
<td>14</td>
<td>1112</td>
<td>1112</td>
</tr>
<tr>
<td>15</td>
<td>1030</td>
<td>1030</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Based on the discussion above, Table 9 summarized the important differences between the raw materials and the isolated lignins. It can be seen a significant difference between the functional groups of the raw materials. The major chemical compound in bioethanol biorefinery residue was found to be cellulosic materials, while low molecular weight lignin-like materials were domain chemical compounds in black liquor. However, isolated lignins from both raw materials and commercial lignins showed minor differences in their chemical structure mostly due to absence of syringyl units.

Table 9. Summary of important bands

<table>
<thead>
<tr>
<th>Sample</th>
<th>Band positions (cm(^{-1}))</th>
<th>Indicating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioethanol biorefinery residue</td>
<td>1030, 1055, 1158</td>
<td>Glycosidic bonds</td>
</tr>
<tr>
<td>Black liquor</td>
<td>1414, 1445, 1492, 1577</td>
<td>Low molecular weight lignin-like compounds</td>
</tr>
<tr>
<td>L1</td>
<td>1326, 1268, 1112</td>
<td>Syringyl, Guaiacyl</td>
</tr>
<tr>
<td>L2</td>
<td>1326, 1268, 1112</td>
<td>Syringyl, Guaiacyl</td>
</tr>
<tr>
<td>L3</td>
<td>1266</td>
<td>Guaiacyl</td>
</tr>
<tr>
<td>L4</td>
<td>1325, 1266, 1112</td>
<td>Syringyl, Guaiacyl</td>
</tr>
</tbody>
</table>

3.2.8 Elemental composition of lignin

The elemental compositions of the four lignin samples can be found in Table 10. Lignin L2 (hardwood lignin) has lowest carbon and highest oxygen content, while lignin L3 (softwood lignin) has highest carbon and lowest oxygen content. This could be related to the number of syringyl groups and consequently to the methoxyl content in lignin molecules (Schorr et al., 2014). Lignins with higher number of methoxyl groups contain lower percentage by weight of carbon and higher percentage by weight of oxygen.
The elemental composition of lignin can be used to predict the empirical formula of each lignin (Table 10). The ratio of elements was calculated by the element percentage divided by the molar mass.

L1 exhibited lowest amounts of sulfur (0.03%) because sulfur was not involved in the bioethanol biorefinery process however it was used (in the form of sulfuric acid) in the isolation process. The highest amount of sulfur was exhibited by both kraft lignins L2 (2.5%) and L3 (1.50%). In literature, the sulfur content for L3 was reported 1.05% (Cateto et al., 2008) and 2.1% (Schorr et al., 2014). L4 (commercial sulfur-free lignin) exhibited a low percentage of sulfur (0.37%). The amount of sulfur in L4 was reported 0.41% (Sahoo et al., 2011b). Therefore, L1 could be also utilized as a sulfur-free lignin for different industrial application where sulfur interfering in the process.

The percentage of nitrogen in each lignin varies due to the differences in source of the plant and additives in commercial lignins (Sahoo et al., 2011b). The lowest amount of nitrogen content was present in the hardwood kraft lignin (0.13%), while L3 (the other kraft lignin) contains the highest nitrogen content (0.64%) in comparison with other lignins. The nitrogen content of L3 was reported 0.48% in literature (Cateto et al., 2008). L4, wheat straw lignin, contains 0.59% nitrogen contents. Sahoo et al., (2011b) reported 0.66% nitrogen content in L4 and they stated that the high percentage of nitrogen in commercial lignins might be due to some added amino compounds for making the lignin compatible with different polymeric systems (Sahoo et al., 2011b). However, whether this nitrogen is present as part of macromolecule structure of lignin cannot be confirm from this work.

3.2.9 Heating value estimation

The energy value of the lignin is an important characteristic since lignin is commonly used in combustion and gasification applications. The energy value of the samples can be predicted from their elemental composition using Dulong’s equation (Equation 5).
A higher proportion of carbon in the lignin molecule is preferable as it will lead to higher energy generation in a combustion process (Protasio et al., 2013). Comparison of the higher heating value among the lignin samples shows that L3 has the highest carbon and lowest oxygen content and consequently the highest energy value, while the lowest carbon and highest oxygen content was found in L2 with the lowest energy value.

Higher H/C ratio gives more reactivity in the fuel and as a result it would be better source for gasification. For instance, cellulose \((C_6H_{10}O_5)_n\) and hemicelluloses \((C_5H_8O_4)_n\) have H/C of 1.67 and 1.60, respectively. In this regard, the molar ratio of H/C for all lignin samples was found to be in the range of 1.13 to 1.20, which indicating that there is not much difference between the H/C ratios of four lignin samples and it is much lower in comparison with cellulose or hemicelluloses.
Table 10. Elemental composition, empirical formula, higher heating value (HHV) and H/C ratio of lignin samples

<table>
<thead>
<tr>
<th>Lignin</th>
<th>C (%)</th>
<th>H (%)</th>
<th>N (%)</th>
<th>S (%)</th>
<th>O (%)</th>
<th>Empirical Formula</th>
<th>HHV (MJ/kg)</th>
<th>H/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>62.36</td>
<td>5.89</td>
<td>0.49</td>
<td>0.03</td>
<td>31.22</td>
<td>C_{5.20}H_{5.89}N_{0.035}S_{0.001}O_{1.95}</td>
<td>24.39</td>
<td>1.13</td>
</tr>
<tr>
<td>L2</td>
<td>59.51</td>
<td>5.79</td>
<td>0.13</td>
<td>2.54</td>
<td>32.03</td>
<td>C_{4.96}H_{5.79}N_{0.010}S_{0.079}O_{2.00}</td>
<td>23.23</td>
<td>1.17</td>
</tr>
<tr>
<td>L3</td>
<td>66.10</td>
<td>6.37</td>
<td>0.67</td>
<td>1.57</td>
<td>25.30</td>
<td>C_{5.51}H_{6.37}N_{0.048}S_{0.049}O_{1.58}</td>
<td>27.22</td>
<td>1.16</td>
</tr>
<tr>
<td>L4</td>
<td>65.41</td>
<td>6.53</td>
<td>0.59</td>
<td>0.38</td>
<td>27.09</td>
<td>C_{5.45}H_{6.53}N_{0.042}S_{0.012}O_{1.69}</td>
<td>26.95</td>
<td>1.20</td>
</tr>
</tbody>
</table>
3.2.10 Total hydroxyl and carboxyl content

Table 11 shows the total hydroxyl and carboxyl content which were determined by $^{31}$P-NMR spectroscopy and titration methods. Cateto, et al., (2008) reported that the values of the total hydroxyl content of four different technical lignins determined by titration and $^{31}$PNMR were in good agreement (Cateto et al., 2008). The $^{31}$P-NMR spectroscopy technique was found to be a very powerful tool for characterization of phenolic hydroxyl (p-hydroxyphenyl, guaiacyl, and syringyl structures), aliphatic hydroxyl and carboxylic acid groups present in the lignin samples (Cateto et al., 2008). In $^{31}$P-NMR spectrum (Figure 12), the signals in the range of 149.2-146.0, 144.3-137.2 and 135.6-133.7 ppm are associated with aliphatic, phenolic and carboxylic acid units, respectively (Monteil-Rivera et al., 2013; Zhang et al., 2013a).

Carboxyl content of L2 was highest (0.90 mmol/g by titration and 0.82 mmol/g by $^{31}$P-NMR) compared to the other lignin samples. Oxidation reactions that may occur during the pulping process cause the lignin structure to acquire carboxyl groups. A study showed that low dosage of oxidant can increase the content of carboxyl in alkali lignin, and high dosage of oxidant may cleave the C-C bond in side chain of phenylpropane unit and further be oxidized into carboxyl (Zhao and Ouyang, 2012). Therefore, carboxyl content can be an estimation of the degradation degree of lignin macromolecule. It can be suggested that L2 is the most degraded from its original form of the four lignins tested in this study.

Both titration and $^{31}$P-NMR results showed that L1 contains the lowest and L2 the highest hydroxyl content in comparison with other lignin samples. The total hydroxyl content of the commercial L3 lignin was in close agreement with values found in the literature which was 7.32 mmol/g by titration and 6.85 mmol/g by $^{31}$P-NMR (Cateto et al., 2008). Number of hydroxyl groups is one of the most characteristic functions in lignin which shows the reactivity in lignin macromolecular chemistry (Cateto et al., 2008). Lignin as a polymer with a fair amount of hydroxyl (phenolic and aliphatic) and carboxyl groups, has the potential to replace polyols in polyurethane production (Pan and Saddler, 2013).
$^{31}$P-NMR results also revealed a significant difference between the number of aromatic hydroxyl groups, while a similar number of aliphatic hydroxyl for lignin samples (Table 11). The differences in the number of phenolic hydroxyl related to the severity of the extraction method. In the kraft and soda processes, β-O-4 and α-O-4 linkages are cleaved and produce non-etherified phenolic hydroxyl groups in lignin. In steam explosion separation, less chemicals are utilized during the process and as a result the lignin undergoes less bond cleavage. In result, L1 shows less phenolic OH group in comparison with kraft and soda lignins.

Table 11. Data obtained for the total hydroxyl and carboxyl content with titration and $^{31}$P-NMR, and total phenolic and aliphatic hydroxyl contents with $^{31}$P-NMR (unit mmol/g)

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Titration</th>
<th>$^{31}$P-NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total OH</td>
<td>Total COOH</td>
</tr>
<tr>
<td>L1</td>
<td>4.02</td>
<td>0.37</td>
</tr>
<tr>
<td>L2</td>
<td>6.50</td>
<td>0.90</td>
</tr>
<tr>
<td>L3</td>
<td>6.32</td>
<td>0.57</td>
</tr>
<tr>
<td>L4</td>
<td>4.25</td>
<td>0.55</td>
</tr>
</tbody>
</table>
3.2.11 Determination of G/H/S ratio by $^{31}$PNMR

The $^{31}$PNMR analysis of lignin samples was carried out to determine the ratio of lignin units of four lignin samples. Table 12 represents the molar ratio of each phenylpropanoid type (G, S and H) in four lignin samples. The ratio of each unit was measured by calculating the area under each band and internal standard. The signals at 143.1-142.4 ppm, 140.0-138.8 ppm, and 138.2-137.2 ppm are attributed to syringyl, guaiacyl and p-hydroxyphenyl units, respectively.

The percentage of phenylpropanoid units in lignin structure is different based on the plants type (softwoods, hardwoods and non-woods) (Telmo and Lousada, 2011). The ratio of phenylpropanoid units in L1 and L4 lignins varies, containing all three precursors. In general, the content of p-hydroxyphenyl in annual crops (L1 and L4) is higher than softwoods and hardwoods. L2 (hardwood lignin) is primarily comprised
guaiacyl and syringyl lignins with minor hydroxyphenyl lignin. L3 (softwoods lignin) contains of guaiacyl with only small amounts of hydroxyphenyl units.

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Syringyl (%)</th>
<th>Guaiacyl (%)</th>
<th>p-Hydroxyphenyl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>26</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>L2</td>
<td>68</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>L3</td>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>L4</td>
<td>51</td>
<td>40</td>
<td>9</td>
</tr>
</tbody>
</table>

3.2.12 \(^1\text{H}\)-NMR spectrometry

Acetylated lignins were analyzed with \(^1\text{H}\)-NMR to determine the content of methoxyl groups, number of aliphatic hydrogens, aromatic hydrogens (free positions on the aromatic ring), aliphatic and aromatic hydroxyl groups in lignin samples by integration of the hydrogen signal of the related region. The \(^1\text{H}\)-NMR spectrum of acetylated lignins is exhibited in Figure 13.
The area of each hydrogen type was obtained by integration of the hydrogen signals in ¹H-NMR spectrum (Gonçalves et al., 2000; Jahan et al., 2012). From the elemental analysis (see Table 10), we found 5.89, 5.79, 6.37 and 6.53 is the total atom ratio of proton in L1, L2, L3 and L4, respectively. The integration of the hydrogen signals (Table 13) results in a total area of 100 arbitrary units which corresponds to the number of protons in the above-given ratio.
Table 13. Area and number of hydrogen in lignin samples obtained from 1H-NMR spectrums

<table>
<thead>
<tr>
<th>Region δ(ppm)</th>
<th>Attribution</th>
<th>L1</th>
<th></th>
<th>L2</th>
<th></th>
<th>L3</th>
<th></th>
<th>L4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Area</td>
<td>Hydrogen</td>
<td>Area</td>
<td>Hydrogen</td>
<td>Area</td>
<td>Hydrogen</td>
<td>Area</td>
<td>Hydrogen</td>
</tr>
<tr>
<td>9.00-12.0</td>
<td>Carboxylic acids and aldehydes</td>
<td>0.08</td>
<td>0.00</td>
<td>0.05</td>
<td>0.00</td>
<td>0.69</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>6.20-7.90</td>
<td>Aromatic region</td>
<td>13.86</td>
<td>0.82</td>
<td>18.78</td>
<td>1.09</td>
<td>22.69</td>
<td>1.45</td>
<td>17.78</td>
<td>1.16</td>
</tr>
<tr>
<td>5.75-6.20</td>
<td>Noncyclic benzylic region</td>
<td>2.06</td>
<td>0.12</td>
<td>0.46</td>
<td>0.03</td>
<td>1.34</td>
<td>0.09</td>
<td>0.30</td>
<td>0.02</td>
</tr>
<tr>
<td>5.20-5.75</td>
<td>Cyclic benzylic region</td>
<td>0.07</td>
<td>0.00</td>
<td>0.65</td>
<td>0.04</td>
<td>3.45</td>
<td>0.22</td>
<td>1.11</td>
<td>0.07</td>
</tr>
<tr>
<td>3.95-5.20</td>
<td>Aliphatic region</td>
<td>10.10</td>
<td>0.59</td>
<td>1.84</td>
<td>0.11</td>
<td>2.01</td>
<td>0.13</td>
<td>3.47</td>
<td>0.23</td>
</tr>
<tr>
<td>3.40-3.95</td>
<td>Methoxyl</td>
<td>35.00</td>
<td>2.06</td>
<td>35.59</td>
<td>2.06</td>
<td>26.40</td>
<td>1.68</td>
<td>33.88</td>
<td>2.21</td>
</tr>
<tr>
<td>2.50-3.20</td>
<td>Aliphatic region</td>
<td>2.47</td>
<td>0.15</td>
<td>5.91</td>
<td>0.34</td>
<td>0.16</td>
<td>0.01</td>
<td>4.77</td>
<td>0.31</td>
</tr>
<tr>
<td>2.10-2.45</td>
<td>Aromatic acetoxy region</td>
<td>11.67</td>
<td>0.69</td>
<td>21.23</td>
<td>1.23</td>
<td>17.31</td>
<td>1.10</td>
<td>18.59</td>
<td>1.21</td>
</tr>
<tr>
<td>1.60-2.10</td>
<td>Aliphatic acetoxy region</td>
<td>21.65</td>
<td>1.28</td>
<td>14.46</td>
<td>0.84</td>
<td>23.80</td>
<td>1.52</td>
<td>16.97</td>
<td>1.11</td>
</tr>
<tr>
<td>0.00-1.60</td>
<td>Nonoxygenated aliphatic region</td>
<td>3.05</td>
<td>0.18</td>
<td>1.03</td>
<td>0.06</td>
<td>2.15</td>
<td>0.14</td>
<td>3.12</td>
<td>0.20</td>
</tr>
<tr>
<td>sum</td>
<td></td>
<td>100.00</td>
<td>5.89</td>
<td>100.00</td>
<td>5.79</td>
<td>100.00</td>
<td>6.37</td>
<td>100.00</td>
<td>6.53</td>
</tr>
</tbody>
</table>
The number of methoxyl groups was calculated from number of hydrogen atoms divided by 3 hydrogens in the methoxyl region (δ 3.40-3.95). Therefore, the number of methoxyl groups was calculated 0.69, 0.69, 0.56 and 0.74 for L1, L2, L3 and L4, respectively. Theoretically, the molecular structure of lignin is composed of hydroxyphenyl structural units which phenol ring attached to a propyl side-chain. Based on the number of methoxyl groups on the phenol ring, the structure is called guaiacyl (1 methoxyl), syringyl (2 methoxyl) and p-hydroxyphenyl (0 methoxyl). The C9-formula of lignin is a combination of these three units. Table 14 represents the approximate C9-formula derived from empirical formula (Table 10) and the ratio of OCH$_3$ for each lignin sample. L2 (hardwood lignin) contains the highest amount of methoxyl groups and L3 (softwood lignin) contains the lowest. C9-formula for L3 (C$_9$H$_{8.53}$N$_{0.078}$S$_{0.080}$O$_{1.85}$(OCH$_3$)$_{1.02}$) was also reported in literature, C$_9$H$_{8.74}$N$_{0.064}$S$_{0.062}$O$_{2.56}$(OCH$_3$)$_{0.77}$ (Cateto et al., 2008) and C$_9$H$_{8.13}$N$_{0.05}$.S$_{0.11}$O$_{2.1}$(OCH$_3$)$_{0.66}$ (Schorr et al., 2014). However, these values are an approximation of C9-formula because technical lignins may contain some impurities such as ash and carbohydrates. Based on the empirical formula, the molecular weight (Mw) of the C9 unit was also determined for each lignin sample.

The ratio of aromatic hydrogens (H$^{ar}$) and aliphatic hydrogens (H$^{al}$) as well as the ratio of phenolic hydroxyl (OH$^{ph}$) and aliphatic hydroxyl (OH$^{al}$) groups was also found through integration of $^1$H-NMR bands from different regions. Aromatic and aliphatic acetoxyl groups were divided by 3 to find the number of aromatic and aliphatic hydroxyl groups. Thus an expanded C9-formula for lignin samples was established as presented in Table 14. In expanded C9-formula, the atom ratio of acidic and aldehyde protons as well as sulfur and nitrogen were not presented because they were negligible in comparison to other groups or atoms.

In alkaline process, phenolic hydroxyl groups are generated by hydrolysis of β-O-4 bond (Zhao and Ouyang, 2012). Phenolic hydroxyl content is increased by increasing the β-O-4 cleavage. Therefore, the number of phenolic hydroxyl in expanded C9-formula of L1 lignin was significantly lower than other lignins, which is correlated with FTIR results.
FTIR spectra showed that the band intensity of in-plane deformation vibration of phenolic OH (1365 cm\(^{-1}\)) for L1 lignin is less than other lignins. Yanez and co-workers (2014) reported the expanded C9-formula of organosolv lignin at different severity (H-factor) (Yáñez-S et al., 2014). The expanded molecular formula of lignin at the highest and lowest severity was \( \text{C}_9\text{H}_{9.98}\text{O}_{1.55}(\text{OCH}_3)_{1.25}(\text{OH}_\text{Ar})_{0.40}(\text{OH}_\text{Alk})_{0.26}(\text{COOH})_{0.036} \) and \( \text{C}_9\text{H}_{9.44}\text{O}_{1.48}(\text{OCH}_3)_{1.52}(\text{OH}_\text{Ar})_{0.39}(\text{OH}_\text{Alk})_{0.33}(\text{COOH})_{0.029} \), respectively.

3.2.13 Double bonds equivalent (DBE)

From C9-formula, the double bonds equivalent (DBE) was also found for each lignin sample. The degree of unsaturation was estimated according to the following equation (Robert et al., 1984):

\[
\text{DBE} = \frac{(2a+2)-b}{2}
\]

DBE-values revealed the number of double bonds, the presence of the aromatic ring together with the degree of inter-unit linkages in the phenylpropanoid lignin units. The calculated DBE in Table 14 shows that isolated lignin from bioethanol biorefinery residue (L1) contains more unsaturated bonds than the kraft and soda lignin samples. The lower DBE of lignin can be attributed to the cleavage of β-aryl ether bonds and to the formation of free phenolic hydroxyl groups (Mansouri and Salvadó, 2006; Robert et al., 1984). The DBE value for milled wood lignin from spruce was reported to be 5.36 by Mansouri et al. (2006). Cracking reactions may occur during pulping treatment of kraft and soda which causes double bonds in the lignin structure (Anglès et al., 2003).
Table 14. C9-formula, expanded C9-formula, double bond equivalent (DBE) and molecular weight for lignin samples

<table>
<thead>
<tr>
<th>Lignin</th>
<th>C9-formula</th>
<th>Extended C9-formula</th>
<th>DBE</th>
<th>Mw</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>C₉H₇.₆₄N₀.₀₆₁S₀.₀₀₂O₂.₅₂(OCH₃)₁.₃₇</td>
<td>C₉H₃.₆₃H₄.₇₀O₁.₂₁(OH)₀.₄₆(OH)₀.₈₅(OCH₃)₁.₃₇</td>
<td>5.₅₀</td>
<td>1₉₈.₄</td>
</tr>
<tr>
<td>L2</td>
<td>C₉H₇.₈₅N₀.₀₁₇S₀.₁₄₄O₂.₇₇(OCH₃)₁.₄₅</td>
<td>C₉H₂.₉₉H₄.₁₁O₁.₃₁(OH)₀.₈₆(OH)₀.₅₉(OCH₃)₁.₄₅</td>
<td>5.₃₅</td>
<td>2₀₅.₀</td>
</tr>
<tr>
<td>L3</td>
<td>C₉H₈.₅₃N₀.₀₇₈S₀.₀₈₀O₁.₈₅(OCH₃)₁.₀₂</td>
<td>C₉H₂.₆₃H₄.₃₁O₀.₂₇(OH)₀.₆₇(OH)₀.₉₂(OCH₃)₁.₀₂</td>
<td>₅.₂₃</td>
<td>₁₇₇.₈</td>
</tr>
<tr>
<td>L4</td>
<td>C₉H₈.₂₅N₀.₀₇₀S₀.₀₁₉O₁.₈₂(OCH₃)₁.₄₁</td>
<td>C₉H₂.₂₂H₄.₅₅O₀.₃₄(OH)₀.₇₇(OH)₀.₇₁(OCH₃)₁.₄₁</td>
<td>₅.₁₇</td>
<td>₁₸₉.₀</td>
</tr>
</tbody>
</table>
3.2.14 Solubility of lignin in alkaline solution

Figure 14 shows the solubility and the pH of the solution of lignin samples in a series of NaOH solutions with different concentrations (0-0.2 mol/L). It can be seen that the dependence of the lignin solubility consists of three portions. The first portion was observed at very low concentration of NaOH (0 – 0.01 mol/L). The solubility was very low in this interval. The intercept shows the solubility of lignin in water. The solubility of L1, L2, L3 and L4 in water is estimated to be 6.7 %, 4.0 %, 4.5 % and 1.5 % respectively. Interval from 0.01 – 0.1 mol/L NaOH is linear for all lignins. The third portion is at 0.1 - 0.2 M NaOH with high to complete dissolution.

Figure 14. Solubility of lignin samples in NaOH
3.2.15 Determination of Molecular Mass Distribution (MMD) of lignin

Molecular masses of the lignin samples were analyzed by alkaline aqueous phase High Performance Size Exclusion Chromatography (HPSEC). One of the advantages of using alkaline eluent is the good solubility of lignin in alkaline solution (Bo et al., 2003). The UV detector was adjusted at 280 nm due to the maximum UV absorption of lignin. This wavelength was used to estimate the molar concentration of the aromatic rings. Poly(styrene sulfonate) sodium (PSS) which is known to exhibit a similar behavior to lignin was used for calibration of the column (González et al., 2000).

Figure 15 shows the molecular weight distributions of sodium polystyrene sulfonates standards (6520, 4230, 1830 and 1100 daltons).

![Figure 15. Molecular weight distributions of standards; Sodium poly(styrene sulfonates) (PSS) with different peak molecular weight of 1100, 1830, 4230 and 6520 daltons](attachment:image.png)
The calibration curve of the standards was obtained for determination of lignin molecular weight (Figure 16).

Figure 16. Calibration curve for the PSS standard solutions

Figure 17a and Figure 17b exhibit the chromatographs of HPSEC and molecular weight distribution of four lignin samples. All chromatographs show that the molecular weight distributions of lignins have a normal curve, while L1 lignin has a bimodal curve, which shows that there are two lignin weight fractions. The fraction with higher molecular weight was appeared out of the column range.
Figure 17. Molecular weight distributions of lignin samples, a) Intensity vs. Time b) Ai (mass fraction) vs. Mi (Molecular weight)

The number and weight average molecular weights and molecular weight distribution were calculated based on the ASTM D5296 –11.

Number – average molecular weight:

\[ M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum_{i=1}^{N} A_i}{\sum_{i}^{N} M_i} = \frac{\sum_{i}^{N} h_i}{\sum_{i}^{N} M_i} \quad (7) \]
Weight – average molecular weight:

\[ M_W = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum_{i=1}^{N} A_i M_i}{\sum_{i=1}^{N} A_i} = \frac{\sum_{i=1}^{N} h_i M_i}{\sum_{i=1}^{N} h_i} \]  \hspace{1cm} (8)

Polydispersity

\[ PD = \frac{M_W}{M_n} \]  \hspace{1cm} (9)

where

- \( n_i \) is the number of molecules of molecular weight \( M_i \)
- \( M_i \) is the molecular weight
- \( A_i \) is the slice area at each interval of molecular weight \( M_i \)
- \( h_i \) is the peak height at each interval of molecular weight \( M_i \)
- \( PD \) is the polydispersity

Table 15 indicates number-average (\( M_n \)), weight-average (\( M_W \)) molecular weights, polydispersity (\( PD \)), number average (\( Dpn \)) and mass average (\( Dpw \)) degree of polymerization for the four lignins. \( Dpn \) and \( Dpw \) were calculated by dividing \( M_n \) or \( M_W \) by the molecular mass of C9-formula of each lignin (Schorr et al., 2014). Molecular weight of lignin has been reported to be dependent on the apparatus and protocol used for its isolation and is thus difficult to compare (Baumberger et al., 2007). The isolated lignin from bioethanol biorefinery residue (lignin L1) has higher molecular weight than other isolated lignins. This is due to less cleavage of ether bonds in the steam explosion and enzymatic hydrolysis process. This agrees with the higher number of phenolic
hydroxyl in kraft and soda lignins (Table 11) in which the cleavage of β-O-4 bonds is occurring in the alkaline pulping process.

Degree of fragmentation during alkaline pulping process may affect the molar mass of lignin. In the kraft process α-aryl and β-aryl linkages are cleaved while in the soda process of non-woody plants mostly α-aryl linkages cleaved and only small quantities of β-aryl linkages cleaved (Tejado et al., 2007). β-aryl cleavage occurred during kraft method due to the more severe conditions used, which causes the appearance of lower Mw species than in soda. Therefore, L2 lignin has been found to have lower average molecular weight than L4 which makes it more valuable in certain applications.

The molecular weight of lignin depends not only on the isolation process of each sample but on plant species (percentage of G/S/H units) (Cazacu et al., 2013). After β-O-4 (the most common bonds between lignin units), C–C bonds are important linkages between the structural units. Among C–C bonds, the most abundant bond is C5 which makes a linkage between aromatic rings (Brunow et al., 1999). These bonds are formed only between guaiacyl units, while it is not possible to form between syringyl units as methoxyl groups are substituted at this position. During pulping processes, C-C bonds are not cleaved due to their higher stability. Therefore, lignins with higher guaiacyl units (softwoods) are expected to have a higher molecular weight than syringyl units (hardwoods and non-woods). Therefore, as expected, the molecular weight of L3 lignin is higher than other L2 and L4 lignin samples.

L1 lignin showed a higher Dpn and Dpw than kraft and soda lignins. The highest mass average degree of polymerization between isolated lignins from pulping processes is for L3 lignin (softwood Kraft lignin). The differences between Dpn and Dpw of lignin samples could be explained by the differences of the plant origins and delignification procedures.
Table 15. The number average (Mn), weight average (Mw) molecular weight, polydispersity (PD), number average (Dpn) and mass average (Dpw) degree of polymerization for the four lignin samples

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Mn (g/mol)</th>
<th>Mw (g/mol)</th>
<th>PD</th>
<th>Dpn</th>
<th>Dpw</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1093</td>
<td>13488</td>
<td>12.34</td>
<td>5.5</td>
<td>68.0</td>
</tr>
<tr>
<td>L2</td>
<td>866</td>
<td>2565</td>
<td>2.96</td>
<td>4.2</td>
<td>12.5</td>
</tr>
<tr>
<td>L3</td>
<td>1191</td>
<td>6096</td>
<td>5.12</td>
<td>6.7</td>
<td>34.3</td>
</tr>
<tr>
<td>L4</td>
<td>1084</td>
<td>5008</td>
<td>4.62</td>
<td>5.7</td>
<td>26.5</td>
</tr>
</tbody>
</table>

3.2.16 Potential applications for lignin from different sources

Industrial applications for isolated lignin samples are summarized in Table 16 based on their relevant physico-chemical properties. It can be seen that the molecular weight of L1 is much higher than other lignin samples. Lignins with high molecular weight are preferred as matrix in polyolefins composites, because higher molecular mass of lignin has better affinity with the matrix (Schorr et al., 2014). Lignins with lower molecular weight, such as L2, L3 and L4, are preferred for phenol-formaldehyde resin due to higher reactivity than lignins with high molecular weight (Mansouri and Salvadó, 2006). Moreover, lignin with lower molecular weight can be utilized in other applications like antioxidants, adhesives and paints (Hussin et al., 2014) while, lignin with high molecular weight shows poor antioxidant activity (El Hage et al., 2012).

Lower percentage of sulfur and ash contents in lignin is preferred for most of the value added applications. For instance, sulfur-free lignins are preferably used in moulded plastics and filler for the polymers in which lower toxic gas emission during processing and disposal is produced (Lora and Glasser, 2002; Sahoo et al., 2011b). Furthermore, sulfur–free lignins can be also used in thermosetting formulations for preparation of resins such as phenolic, epoxy and isocyanate (Glasser, 1989; Kelley et al., 1989). In
addition, low sulfur and ash content is more favorable for synthesis of polymers and low-molecular weight substances (Vishtal and Kraslawski, 2011b). Therefore, L1 and L4 (sulfur-free lignins) have great potential application in plastic and resin industry where sulfonated lignins are limited to be used.

Nitrogen in lignin samples is present as proteins or amino acids (organic materials) which is generates from the original source (Niemelä, 1990; Veverka and Nichols, 1992). Nitrogen-containing substances from lignin can be removed by treatment with proteolytic enzymes such as proteases (Vishtal and Kraslawski, 2011b). The high percentage of nitrogen in commercial lignins might be due to some amino compounds which is added to the lignin to make it compatible with different polymeric systems (Sahoo et al., 2011b). The percentage of nitrogen in L1 is high which might be due to the remaining enzymes in the hydrolysis process.

It is important to note that lignin with a fair amount of phenolic hydroxyl, aliphatic hydroxyl and carboxyl groups has great potential to replace polyols in polyurethane production (Pan and Saddler, 2013). For instance polyurethane foam was prepared from organosolv lignin and kraft lignin which contains 5.64 mmol/g and 8.41 mmol/g hydroxyl, respectively (Pan and Saddler, 2013). Polyurethane film was prepared with consistent properties from 50% organosolv lignin (total hydroxyl content of 5.38 mmol/g) and polyethylene glycol as co-polyol (total hydroxyl content of 5 mmol/g) with using a catalyst for polymerization (Ni and Thring, 2003). Therefore, L2 and L3 with high number of hydroxyl groups can be used as polyol in synthesis of polyurethane and epoxy resins. Although, chemical modification such as oxypropylation with alkylene oxide improve the accessibility of hydroxyl groups of lignin macromolecule (Cateto et al., 2009; Lora and Glasser, 2002), L1 with relatively high Mw may not be suitable for this application.

Polymerization reaction of formaldehyde at free positions of phenol takes place through electrophillic substitution during the synthesis of phenol-formaldehyde resin. In lignin, both C3 and C5 positions are free in p-hydroxyphenyl units, while guaiacyl units have a free C5 position in the ring. In syringyl units both C3 and C5 are linked to a methoxy
group, resulting in low reactivity of lignin with formaldehyde. Therefore, lignin with p-hydroxyphenyl or guaiacyl units must be more suitable for phenol-formaldehyde formulations. However, it is very important to note that the quantity of phenolic hydroxyls in lignin molecule activate the free ring positions, while these phenolic hydroxyl can also promote non-covalent interaction between lignin units making lignin stiff macromolecule which can decrease of final properties (Tejado et al., 2007). L1 contains high number of p-hydroxyphenyl and guaiacyl units, however the number of phenolic hydroxyl is lower that other lignin samples. On the other hand, the number of phenolic groups in L2 is higher than other lignins, but the syringyl is the dominate unit. In result, the reactivity of both L1 and L2 lignins low with formaldehyde. L3 (mainly composed by guaiacyl units) presents high quantities of free positions and phenolic hydroxyl in comparison with other lignin samples. Therefore, L3 may have higher reactivity towards electrophilic substitution reactions and it would be more appropriate for phenolic resins than the other lignin samples (Tejado et al., 2007).
Table 16. Summarized physico-chemical properties of lignin samples with potential applications

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Mw (g/mol)</th>
<th>Ash (%)</th>
<th>Impurities in the raw material</th>
<th>S:G:H</th>
<th>$\text{OH}^\text{ph}$ (mmol/g)</th>
<th>$\text{OH}^\text{al}$ (mmol/g)</th>
<th>Sulfur (%)</th>
<th>Nitrogen (%)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>13488</td>
<td>0.45</td>
<td>Cellulosic materials</td>
<td>26:31:44</td>
<td>1.51</td>
<td>2.22</td>
<td>0.03</td>
<td>0.49</td>
<td>Filler for plastics and polymer, composites</td>
</tr>
<tr>
<td>L2</td>
<td>2565</td>
<td>1.54</td>
<td>Low Mw lignin-like materials</td>
<td>68:31:1</td>
<td>6.91</td>
<td>2.35</td>
<td>2.54</td>
<td>0.13</td>
<td>Phenolic, polyurethane and epoxy resins, antioxidants, adhesives and paints, thermoplastic</td>
</tr>
<tr>
<td>L3</td>
<td>6096</td>
<td>4.25</td>
<td>NA</td>
<td>0:95:5</td>
<td>4.00</td>
<td>2.59</td>
<td>1.57</td>
<td>0.67</td>
<td>Phenolic, polyurethane and epoxy resins, antioxidants, adhesives and paints, thermoplastic</td>
</tr>
<tr>
<td>L4</td>
<td>5008</td>
<td>1.38</td>
<td>NA</td>
<td>51:40:9</td>
<td>2.28</td>
<td>2.47</td>
<td>0.38</td>
<td>0.59</td>
<td>Filler for plastics and polymers, Phenolic, resins, antioxidants, adhesives and paints</td>
</tr>
</tbody>
</table>
3.3 Conclusions

Lignin is produced as by-product in pulping industries as well as biorefineries. The physico-chemical properties of isolated lignins from different sources were not the same for all lignins. Industrial applications for technical lignins are dependent on relevant physico-chemical properties. Therefore it is important to be cognizant of the variation in industrial lignin sources when selecting the best material for use in specific applications. Approximately, 38 %w/w lignin was isolated from bioethanol biorefinery residue, and 27 %w/w lignin was isolated from kraft black liquor. Isolated lignins were characterized and compared with two commercial lignins; L3, pine kraft lignin and L4, wheat straw soda lignin. It was found that the elemental composition varied in all lignin samples. L3 exhibited the highest carbon content and L2 exhibited the lowest carbon content. Sulfur content in kraft lignins (L2 and L3) exhibited higher percentage in comparison with L1 (steam explosion lignin) and L4 (soda lignin) samples. Low sulfur content is more favorable for lignin in polymer industry due to less emission of toxic gases during the process. FTIR spectrum showed the significant differences between the impurities of two raw materials; cellulosic materials in bioethanol biorefinery residue and low molecular weight lignin-like compounds in kraft black liquor. Molecular weight of L1 was obviously higher than other lignins due to less harsh chemical processes for the isolation of lignin. L1 with higher molecular weight and lowest sulfur content than other lignins is recommended for use in polymer blends and composites. On the other hand, the number of hydroxyl in L2 is relatively high and its molecular weight is lowest in comparison with other lignins. Therefore, L2 is suggested for use in resin industry specifically for polyurethane synthesis. High phenolic hydroxyl content, free substitutions and lower molecular weight is required for phenol-formaldehyde resin formulation. From these criteria, L3 appears to be the better than other lignins for the production of phenol-formaldehyde resins. L4 as sulfur-free lignin has good potential to be used as filler in plastics/polymers industry and as phenol replacement for making phenol-formaldehyde resins.
CHAPTER 4 Thermal Characteristics of Lignin Residue from Industrial Processes

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Authors: Sameni, J., Krigstin, S., dos Santos Rosa, D., Leao, A., Sain, M.,

4.1 Introduction

Lignin is one of the most abundant natural substances in the world, and it is produced as a by-product in pulp mills and cellulosic ethanol biorefineries. Although the major portion of lignin is burned to produce energy in recovery boilers (Mohan et al., 2006), there is potential to utilize it in higher-value applications (Vishtal and Kraslawski, 2011a). The use of lignin is limited for value-added products due to its high impurities, which are generated during the extraction process. Methods exist for the isolation of lignin; however, these processes add cost and complexity to the lignin recovery process. Recently, isolated lignin has been used for conversion into value-added products, such as phenolic resins (Alonso et al., 2004; Khan et al., 2004b; Sarkar and Adhikari, 2001a; Tejado et al., 2007) and polymer blends (Cazacu et al., 2004; Gosselink et al., 2004c; Kadla et al., 2002). Therefore, it of interest to understand the differences between various industrial lignins as they are generated by their original extraction process as compared to their isolated form.

Technical lignins are different from each other to a significant extent because they originate from different sources and separation processes such as kraft, soda, steam explosion, organosolv, and hydrolysis (Vishtal and Kraslawski, 2011a). The kraft process is a prominent method for making pulp and produces the largest volume of lignin. In this process, α-aryl and β-aryl ether bonds in lignin are cleaved by alkaline hydrolysis (in a mixture of sodium hydroxide and sodium sulfide) in which 90% of the lignin is removed before the bleaching process (Chakar and Ragauskas, 2004). The generated aqueous solution, which is called black liquor, contains ligneous material (30 to 45 wt. %), saccharinic acids (25 to 35 wt. %), formic and acetic acid (10 wt. %), extractives (3 to 5 wt. %), sodium (17 to 20 wt. %) and sulfur (3 to 5 wt. %) (Wallberg et
In the steam explosion process, biomass is fractionated for producing cellulosic ethanol (Doherty et al., 2011). In the pretreatment step of the process, biomass is exposed to pressurized steam, followed by depressurization. This rapid change in pressure results in the hydrolysis of the hemicelluloses and the cleavage of lignin-hemicellulose bonds (Li et al., 2007). Therefore, the lignocellulosic structure breaks down, and as a result, the lignin is partially depolymerized while the hemicellulose can be readily hydrolyzed (Cara et al., 2006). The removal of hemicellulose increases the average pore size of the substrate and thus enhances cellulose degradation by making more surface area accessible to enzymes (Hendriks and Zeeman, 2009; Taherzadeh and Karimi, 2008).

There are a number of methods for isolating lignin from the industrial residue stream. The LignoBoost® process is used to remove lignin from concentrated kraft black liquor by acidifying it with carbon dioxide (Beis et al., 2010). When carbon dioxide is absorbed into the alkaline black liquor, the pH is decreased, and the lignin becomes hydrophobic and precipitates. The lignin is then dewatered to 65%, and after filtration, the filter cake is re-dispersed and acidified with sulfuric acid. In another technique (Abacherli and Doppenberg, 2001), lignin is precipitated from the black liquor solution by reducing the pH while at room temperature; subsequently, the mixture is heated to about 70 to 80°C to turn it from a gelatinous form into a filterable form. Lignin is separated by filtration, washed with water, and dried in an oven.

The ash content of herbaceous plants is much higher than that of wood (usually less than 1%) (Pan and Sano, 2005). The major elements in wood ash are calcium, potassium, and magnesium, while sodium and silicon are present in relatively smaller amounts (Misra et al., 1993). Typically, herbaceous plants have much higher Si content. For instance, the ash of wheat straw and rice straw contain 9.6% and 16.5% silicon, respectively, while aspen and white oak wood contain only 0.1% silicon (Misra et al., 1993; Pan and Sano, 2005). However, the sources and the amounts of minerals in industrial lignins depend more on the extraction process than the plant species. For instance, alkaline pulping processes, such as kraft, produce lignin with high ash content (43.6%), while autohydrolysis (1.2%) and organosolv (0.1%) techniques result in low
ash content (Zabaleta, 2012). The sources of these minerals can be the various chemicals used in the pulping process or metallic matter entering the process from piping and machinery (Zabaleta, 2012).

In this work, lignin was isolated from black liquor and bioethanol biorefinery residue to elucidate the differences in behavior of the industrial lignin materials compared to that of their isolated forms. The ash content was also analyzed to determine the chemical composition of mineral impurities in the original and isolated lignin.

4.2 Experimental

4.2.1 Materials

Two industrial lignins and two commercial lignins were used in this study. L1 was derived from a bioethanol biorefinery residue (L1-Orig.), which uses hardwood/non-wood biomass; L2 was isolated from kraft black liquor (L2-Orig.) which was obtained from a pulp mill utilizing eucalypt species. Commercial lignins were L3 (Indulin AT, softwood kraft pine lignin supplied by Westvaco Co.) and L5 (Protobind 1000, non-wood soda lignin supplied by ALM Private Limited). L3-I and L5-I were isolated from L3 and L5.

4.2.2 Lignin Isolation

Lignin samples were isolated using the Abacherli and Doppenberg (Abacherli and Doppenberg, 2001) method. Briefly, lignin was dissolved in 0.5 M NaOH and filtered with a Büchner funnel with a paper membrane filter (1-µm mesh). Lignin was then precipitated with sulfuric acid to a pH of 2 to 3 and heated to 70 to 80 °C. The resulting precipitate was filtered and subsequently washed several times with water at 50 to 60 °C, followed by washing with water at room temperature. The material was dried at 50 °C overnight.
4.2.3 Ash Content Determination

The ash content of the samples was gravimetrically determined after incineration at 525 °C or 900 °C (TAPPI method T 211 om-93, 1999). About 0.5 g of moisture-free sample was weighed and placed in a muffle furnace at 525±25 °C (or 900±25 °C) for 4 h. The temperature was increased to 250 °C slowly so that the sample carbonized without flaming. At the conclusion of heating, samples were placed in a desiccator and cooled to room temperature prior to weighing. Samples were white/gray in color on completion of the heating cycle. Samples were weighed on an analytical balance to the nearest 0.1 mg. The ash content was determined as follows:

\[
\text{Ash Content (\%) = } \left( \frac{\text{mass of ash}}{\text{mass of oven dried sample}} \right) \times 100
\]

(10)

4.2.4 Energy-Dispersive X-Ray Spectroscopy (EDS)

To elucidate the nature of the ash, a scanning electron microscope (SEM) equipped with an energy dispersive spectrometer (EDS) was employed. A thin layer of ash was carefully sprinkled on to double-sided carbon tape mounted on SEM aluminum stubs. All samples were sputter-coated and imaged using a JEOL (JSM-6610LV) scanning electron microscope equipped with a backscattered electron detector in low-vacuum mode (Johnson et al., 2010). Each ash sample was characterized by examining all the ash particles observed within the whole image. EDS analysis detects signals from elements with atomic number equal or greater than six (Kutchko and Kim, 2006) and provides a qualitative analysis (Ozturk et al., 2014).

4.2.5 X-ray Diffraction (XRD)

XRD patterns were collected on a Philips (PW1830) diffractometer (40 kV, 40 mA) equipped with a Ni filter, using Cu Kα radiation (λ =1.54 Å). Scans were performed from 10° to 50° with 0.02° increments at 2 s per step. Identification of chemical compounds was performed using X’Pert Highscore Software (ver. 2.2) and the ICDD database.
4.2.6 Thermogravimetric Analysis (TGA)

About 12 to 14 mg of lignin sample was weighed into a standard ceramic crucible and placed in a NETZSCH thermogravimetric analyser (Model STA 449F3). Heating was performed from room temperature to 1100 °C at a rate of 10 °C min\(^{-1}\). The test was carried out under an argon atmosphere with a flow rate of 20 mL min\(^{-1}\). A curve of weight loss against temperature was obtained from the instrument.

4.2.7 Differential Scanning Calorimetry (DSC)

DSC of lignin samples was performed with a TA instrument-DSC Q200. The results were processed using “Universal 4.2E TA” software. Each sample (2 to 5 mg) was weighed into a standard aluminum pan (40 µL) and heated under a nitrogen atmosphere with a flow rate of 15 mL/min. The sample was initially heated to 200 °C at a heating rate of 10 °C/min. Next, the sample was cooled down to -10 °C with a cooling rate of 30 °C/min. Finally, the sample was reheated to 200 °C with a heating rate of 10 °C/min. The initial heating and cooling cycle was carried out to clear the thermal history of the sample to eliminate the endothermic enthalpy relaxation that usually affects the \(T_g\) determination (Poursorkhabi et al., 2013; Rials and Glasser, 1984). The \(T_g\) value of each sample was measured from the last heating cycle.

4.3 Results and discussion

4.3.1 Ash Analysis

The ash or inorganic content of the original materials and isolated lignins can be seen in Table 17. The ash content of sample L1 represented a fairly small proportion of the material (2.31%) because the bioethanol biorefinery process used steam and enzymes to extract the carbohydrates, which left behind a relatively chemical-free residue. On examination of the EDS spectrum (Figure 18a), it can be seen that its ash contains K, Na, Al, S, and silica. Wood in general contain very little silicone (Baxter et al., 1998); therefore, the presence of silica can be attributed to the wheat straw used in the
bioethanol biorefinery process (Table 17). The ash content of wheat straw is much higher than that of wood. The ash content of wood is usually less than 1%, while the ash content of wheat straw is about 9.6%, of which 76% is silica (Pan and Sano, 2005). L1 contained 0.45% ash, which was the lowest ash among the lignin samples. It has been reported that the lignin produced from organosolv techniques contains 0.1% ash contamination (Zabaleta, 2012).

The ash content of L2-Orig was extremely high and represented approximately 36% of the material. This can be attributed to high amounts of sodium and sulfur used in the kraft process (Wallberg et al., 2003). The ash content of black liquor has been reported to be as much as 30% (Mansouri and Salvadó, 2006). It has been reported that kraft lignins contain 1.5 to 3.0% sulfur, some present as elemental sulfur and some organically bound to the lignin (Gellerstedt and Lindfors, 1984). Normally, inorganic chemicals are recovered and re-used in the process and the lignin is burned as a fuel. If the black liquor is not processed through the recovery system, then there will be a large proportion of these chemicals left in the liquor, as evidenced by the high ash (inorganic) content of L2. The elemental composition of L2 ash can be seen in Figure 18b, showing the presence of K, Na, Al, S, and Si in both the original and isolated lignins.

In both L1-Orig and L2-Orig, the inorganic content was reduced after the isolation process. The ash content of L2-Orig was reduced from 36.29% to 1.54%, whereas the ash content of the original L1-Orig was reduced to 0.45% from 2.31%. For the L2-Orig sample, possible contaminants, such as sodium sulfate and sodium sulfide, would be solubilized in the sodium hydroxide used in the Abacherli and Doppenberg (Abacherli and Doppenberg, 2001) method and removed from the sample during isolation. It can be seen in both the L1 and L2 samples that sulfur was present and may be due to the sulfuric acid used in the isolation process. It is important to note that the ash content in the L1-Orig (steam-exploded process) was much lower than that in the L2-Orig (kraft process).

L3 is commercial kraft lignin made from pine species isolated through an acid hydrolysis process (Beis et al., 2010). This commercial lignin had a relatively high ash content
(4.25%). The percentage ash of the commercial L3 was reported by (Cateto et al., 2009) as 3%. The ash content may be attributed to the sodium salts and the chemically bonded sulfur from the kraft process. L5 is also a commercial lignin, which is derived from wheat straw through a soda process and acid precipitation technique. The ash content of L5 (soda lignin) was 2.04%, which was lower than the ash content of L3 (kraft lignin). The EDS spectra (Figure 18c and Figure 18d) showed the presence of K, Na, Al, Si, and S in both commercial samples. The ash and silicate contents of the wheat straw soda lignin were reported by (Gosselink et al., 2004b) as 1.9% and 0.7%, respectively.

The moisture content of all air-dried isolated lignins was less than 2.5% due to lignin’s low affinity to moisture caused by its hydrophobic structure. Air-dried sample of both L1-Orig and L2-Orig contained more moisture than their isolated forms. Lignin with higher impurities contained more moisture content, which indicated that the impurities contained hydrophilic compounds.

Table 17. Ash content and moisture content of lignin samples

<table>
<thead>
<tr>
<th>Lignin sample</th>
<th>Ash content ±SD (%)</th>
<th>Moisture content ±SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-Orig</td>
<td>2.31±0.07</td>
<td>3.32±0.22</td>
</tr>
<tr>
<td>L2-Orig</td>
<td>36.29±0.29*</td>
<td>8.05±0.06</td>
</tr>
<tr>
<td>L1</td>
<td>0.45±0.09</td>
<td>2.50±0.56</td>
</tr>
<tr>
<td>L2</td>
<td>1.54±0.13</td>
<td>1.13±0.27</td>
</tr>
<tr>
<td>L3</td>
<td>4.25±0.08</td>
<td>1.67±0.32</td>
</tr>
<tr>
<td>L5</td>
<td>2.04±0.12</td>
<td>2.39±1.33</td>
</tr>
</tbody>
</table>

* at 900°C calcium carbonate is converted to calcium oxide.
Figure 18. EDS analysis of lignin ash

Figure 19 shows the X-ray diffraction patterns of lignin ashes. X-ray diffraction scans were run to identify the chemical compounds in each lignin ash. In the XRD scans, the sharp lines matched the spectra of the compounds in the database. In our study, no chemical compound was identified from the XRD pattern of the L1-Orig (Figure 19a) based on the ICDD database. Analysis of the L2-Orig indicated that the ash is a mixture of inorganic compounds. However, the XRD spectrum of the L2 ash clearly identified sodium sulfate and silicate in the sample. The XRD spectra showed that the main inorganic compound in L3 lignin was sodium sulfate, while sodium sulfate and silicon dioxide were the two major compounds in L5 specimen (Figure 19c and Figure 19d).
4.3.2 Thermogravimetry Analysis

The thermal characteristics of lignin can be determined using TGA. Figure 20 shows the mass loss of the original and isolated lignins over the temperature range of 30 to 1100°C. The materials were assessed for thermal degradation (on-set temperature), % mass loss between 200 and 600 °C, and percentage of charred residue remaining at 1000 °C (Table 18). The negligible mass loss below 100 °C was a consequence of water loss.

It can be seen in Figure 20 that the TGA curves of all isolated lignins showed very similar thermal behavior. Their thermal degradation began at 200 to 220 °C, which demonstrated that they are thermally stable below 200 °C. Derivative thermograms
(dTGs) of isolated lignins clearly showed that the thermal activities occurred over the temperature range of 200 to 600 °C. There was an initial rapid degradation until about 350 °C, followed by a much slower rate of degradation, which continued to about 600 °C. L3 exhibited the lowest percentage weight loss (about 54.0%), while the weight loss of other lignins was between 59.0 and 61.0%. The charred residue of isolated lignins was in a range of 37 to 45%. The charred residue of L3 (softwood) was the highest, and that of L5 (non-wood) was the lowest among the lignin samples.

The TGA curves of industrial lignins (L1-Orig and L2-Orig) showed different thermal behaviors. The earlier on-set to thermal degradation for the L1-Orig could be due to the presence of carbohydrates, which normally show an on-set of degradation below 200 °C. The larger proportion of mass loss (68.5%) or volatilization of material between 200 and 600 °C can be attributed to the inclusion of carbohydrates in the residue of the L1-Orig. In addition, the lower char of the L1-Orig (28.1%) in comparison with L1 (38.1%) demonstrated that the majority of components were volatilized, thereby indicating that materials such as cellulose and hemicelluloses were present.

The L2-Orig sample demonstrated a different thermal degradation pattern, starting the degradation at a lower temperature (100 °C) than the L1-Orig (160 °C). The thermogram of the L2-Orig showed a smaller initial degradation (26.0%) in the 200 to 600 °C range, followed by a gradual degradation from 700 to 900 °C. The peak of the first degradation in L2-Orig was shifted to lower temperature due to catalytic effect of inorganics. In the L2 sample, many of the inorganics had already been removed; this can explain the lack of a two-step degradation.

Commercial lignins L3 and L5 showed no differences in the thermal degradation of their original and isolated forms. The low percentages of ash (sodium sulfate and silica) in both lignins have no effect on their thermal properties. Differences in the pyrolysis range of commercial lignins (e.g., L3) have been observed by Beis et al. (2010). The results from their TGA analysis showed the same thermal degradation response as was observed in our work. Thus, it appeared that the isolation method removed impurities from the raw lignin, which caused the observed differences in the thermal behavior. The
impurities in L1 and L2, which are removed by the isolation process, resulted in lignin with the same characteristics as the commercial lignin.

The charred residue of L3 at 1000 °C was about 45%, while for the other samples, it was about 37 to 40%. Above 800 °C, the unvolatilized portion of lignin remains as charred residue due to the formation of highly condensed aromatic structures (Sun et al., 2000; Tejado et al., 2007). The amount of remaining char was inversely proportional to the amount of hydroxyl, methoxyl, and carboxyl groups (Jakab et al., 1995). In other words, higher percentages of charred residue are generated when there is a lower proportion of these functional groups. In addition, the percentage of remaining char directly proportional to the percentage of carbon content in the lignin sample (see Table 10 and Table 18).

![TGA thermogram of original and isolated lignin samples](image)

Figure 20. TGA thermogram of original and isolated lignin samples
Table 18. Onset temperature, degradation temperature, and percentage of charred residues of original and isolated lignins

<table>
<thead>
<tr>
<th>Lignin sample</th>
<th>Onset Temp. (°C)</th>
<th>Degradation 200-600°C (%)</th>
<th>Charred residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-Orig</td>
<td>160</td>
<td>68.5</td>
<td>28.1</td>
</tr>
<tr>
<td>L1</td>
<td>200</td>
<td>61.0</td>
<td>38.1</td>
</tr>
<tr>
<td>L2-Orig</td>
<td>100</td>
<td>26.0</td>
<td>24.0</td>
</tr>
<tr>
<td>L2</td>
<td>200</td>
<td>59.0</td>
<td>40.0</td>
</tr>
<tr>
<td>L3</td>
<td>220</td>
<td>50.0</td>
<td>46.0</td>
</tr>
<tr>
<td>L3-I</td>
<td>220</td>
<td>54.0</td>
<td>45.0</td>
</tr>
<tr>
<td>L5</td>
<td>200</td>
<td>61.0</td>
<td>37.1</td>
</tr>
<tr>
<td>L5-I</td>
<td>200</td>
<td>61.0</td>
<td>37.0</td>
</tr>
</tbody>
</table>

4.3.3 Glass Transition

The DSC results of the original and isolated lignins from the third cycle are shown in Figure 21. The glass transition temperature ($T_g$) and the on-set temperature ($T_d$) of isolated lignin were in the range of 130 to 190 °C and 119 to 175 °C, respectively. This wide range demonstrated that there were differences among the lignins in terms of flexibility and stiffness at elevated temperatures, which is important in industrial applications. The $T_g$ value can be explained by various molecular factors, such as interchain hydrogen bonding, crosslinking density, rigid phenyl groups, and molecular mass (Heitner et al., 2010). Glass transition temperature, molecular weight and polydispersity correlation for a series of lignin samples was studied by Schmidl (1992). The glass transitions of lignin samples were very broad (ranged from 130 to 170 °C), which reflect the effect of differences in pulping conditions on the molecular weight, and it was linearly correlated with polydispersity of the molecular weight (Schmidl, 1992). On the other hand, the char residue is increased with increasing molecular weight. This
effect was explained by Sun et al. (2000) in terms of variation in structure and an increasing degree of branching and condensation of lignin structures (Sun et al. 2000). Therefore, based on the literature, the glass transition is directly proportional to the char residue. We can see this behavior with two kraft lignins (L2 and L3), where the char residue and glass transition of L3 is higher than L2, (molecular weight of softwood lignin is greater than hardwood lignin (Tejado et al. 2007)). However, this trend may not be consistent for all lignins, because both $T_g$ and char residues also depend on the plant source and extraction conditions.

![DSC curves of lignin samples](image)

**Figure 21.** DSC curves of lignin samples

Table 19 shows the $T_g$ and $T_0$ of the original and isolated lignin samples. $T_g$ and $T_0$ were clearly lower in L1-Orig and L2-Orig than the L1 and L2, respectively. Higher amounts of impurities resulted in larger differences in glass transition and on-set temperatures. The $T_g$ values for various lignins have been reported in the literature in the range of 90 to 180 °C (Glasser Wolfgang and Jain Rajesh, 1993; Tejado et al., 2007), with higher values corresponding to softwood lignins. The $T_g$ and $T_0$ of L1 were very similar to those
of the L3. It seems that the L1 and L3 lignins behaved quite similarly once the carbohydrate contaminants were removed through the isolation process. The low $T_g$ of L2-Orig suggested that there may be other polyphenolic macromolecules, in addition to lignin, present in the sample (Sahoo et al., 2011b). L2 remained quite different from the other isolated lignins, with lower $T_g$ and $T_0$.

Table 19. Onset temperature and glass transition temperature for original and isolated lignin samples

<table>
<thead>
<tr>
<th>Lignin sample</th>
<th>On-set Temp. ($T_0$) (°C)</th>
<th>Glass Transition Temp. ($T_g$) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-Orig</td>
<td>136</td>
<td>149</td>
</tr>
<tr>
<td>L1</td>
<td>150</td>
<td>159</td>
</tr>
<tr>
<td>L2-Orig</td>
<td>96</td>
<td>110</td>
</tr>
<tr>
<td>L2</td>
<td>119</td>
<td>130</td>
</tr>
<tr>
<td>L3</td>
<td>144</td>
<td>158</td>
</tr>
<tr>
<td>L3-I</td>
<td>150</td>
<td>162</td>
</tr>
<tr>
<td>L5</td>
<td>164</td>
<td>189</td>
</tr>
<tr>
<td>L5-I</td>
<td>175</td>
<td>190</td>
</tr>
</tbody>
</table>

4.4 Conclusions

One of the main differences between industrial and commercial lignins can be found in their impurities. The impurities in industrial lignins depended on the extraction process and the source of the lignocellulosic material. The inorganic elements in industrial lignin were reduced after the isolation process. Industrial processes such as kraft generate more impurities when compared to steam explosion. Extracted lignin from wheat straw
contained more silicon than hardwood and softwood lignins. The thermal decomposition and percentage of the charred residue of isolated lignins were slightly different from each other. There is a direct relationship between the percentage of remaining char and the percentage of carbon content in the lignin samples. However, the percentage of charred residue in industrial lignins was much higher than that in isolated lignins. The glass transition temperature of lignin samples is reduced with higher percentages of impurities. There was a wide range of $T_g$ (130 to 190 °C) for isolated lignins. Industrial lignins, which have lower costs, can be beneficially utilized once their important characteristics are known. This elemental and thermal analysis of two industrial lignins suggested that steam-exploded lignin may have higher potential for economic return, for instance in carbon fiber production, in comparison with kraft lignin due to its lower inorganic content.
CHAPTER 5 Solubility of lignin and lignin acetate in organic solvents

5.1 Introduction

Today, lignin is isolated from low quality residue in pulping industries and biorefineries for increased profitability (Doherty et al., 2011). However, a major portion of isolated lignin is burned as fuel and a small portion is utilized for value added products. Unknown molecular structure, variable physico-chemical properties and broad molecular weight distribution makes it less applicable in many areas (Lora and Glasser, 2002). For instance, solubility of lignin in organic solvents is still not clear and makes it limited for utilization as a value added product. Solubility of lignin in organic solvents depends on many variables such as lignin structure, molecular weight and presence of hydrophilic moiety in the lignin molecule (Shukry et al., 2008). Acetylation is a technique that can improve the solubility of lignin in organic solvents (Olarte, 2011), but it increases the cost of the lignin raw material as well. Understanding of the solubility of lignin (or acetylated lignin) in organic solvents helps to utilize lignin for high value added products, such as lignin-based micro/nanoparticles.

Isolated lignin from different processes can be applied to produce lignin micro/nanospheres by solvent evaporation technique for controlled release of agricultural actives (Asrar and Ding, 2010). Solvent evaporation and solvent displacement techniques are common methods for preparation of polymer micro/nano-dispersions (Freiberg and Zhu, 2004; Lassalle and Ferreira, 2007). In these methods, particles are formed in uniform spherical units with low polydispersity index. First, the polymer is dissolved in an organic solvent and then intermixed with surfactant aqueous solution. Microparticles are formed in spherical shape after the organic solvent is removed from the emulsion. Solvents with low solubility in water and lower boiling point than water are used in the solvent evaporation technique, while solvents with high solubility in water are used in solvent displacement technique (Astete and Sabliov, 2006). Therefore, solubility of lignin in a suitable organic solvent (such as ether,
dichloromethane, ethyl acetate, and acetone) is needed for producing lignin micro/nanospheres.

5.1.1 Lignin dissolution behavior

Lignin dissolution has been of interest for a long time and its solubility behaviors in different organic solvents have been characterized throughout the years (Cybulska et al., 2012; Ni and Hu, 1995; Quesada-Medina et al., 2010; Shukry et al., 2008; Wang et al., 2011; Zhang and LeBoeuf, 2009). In general, the dissolution of a polymer into a solvent involves chain disentanglement and solvent diffusion when polymer contacts with a thermodynamically compatible solvent (Miller-Chou and Koenig, 2003). Lignin macromolecules contain free space in the form of holes and channels which solvent molecules can penetrate.

The solubility of different lignin samples in a series of organic solvents is discussed in this chapter to investigate the effect of molecular weight, chemical structure and functional groups on the solubility of lignin and lignin acetate in different organic solvents.

5.1.2 Solubility parameters

Solubility parameters are useful to understand the compatibility of polymers, swelling of cured elastomers by solvents, chemical resistance, permeation rates of solvents, and also to characterize the surfaces of fibers, pigments, and fillers (Hansen, 2000). Therefore, the usefulness of lignin in many applications is critically dependent on the solubility parameter. The solubility parameter is related to other physical properties, such as wettability, surface tension, the ultimate strength of materials, and the glass transition temperature of the polymer. Therefore, an estimation of the solubility parameters can often be useful tool for predicting performance and physical properties of lignin. In this chapter, the solubility parameters will be applied to explain the effect of molecular weight, chemical structure and functional groups of four lignin samples on the solubility of lignin and its derivatives in different organic solvents.
5.1.3 Thermodynamics background

The solubility of a polymer in various organic solvents can be predicted by its chemical structure. It is well known that a polymer will dissolve in solvents with similar solubility parameters. This principle is also known as ‘like dissolves like’. Dissolution of a polymer in a solvent is governed by the free energy of mixing (Hansen, 2000).

\[ \Delta G_m = \Delta H_m - T\Delta S_m \]  \hspace{1cm} (11)

where;

\( \Delta G_m \) is the Gibbs free energy change on mixing (J/mol)

\( \Delta H_m \) is the enthalpy change on mixing (J/mol)

\( T \) is the absolute temperature (K)

\( \Delta S_m \) is the entropy change on mixing (J/K.mol)

When the value of the Gibbs free energy change on mixing is negative the mixing process will occur spontaneously. Otherwise, two or more phases may appear from the mixing process. Dissolution of high molecular weight polymer, such as lignin, is normally associated with a small positive entropy change, thus, the enthalpy term is an important factor in determining the sign of the Gibbs free energy change.

5.1.4 Solubility parameter theory

The solubility of a polymer in organic solvents can be explained by the solubility parameter (\( \delta \)-value) theory, which is a useful prediction for non-polar and slightly polar polymers (Quesada-Medina et al., 2010). According to Hildebrand’s theory, the solubility of a polymer in a solvent is defined as the square root of the cohesive energy density (Hildebrand and Scott, 1950):
\[ \delta = \sqrt{\frac{E}{V_m}} = \sqrt{\frac{\Delta H_{vap} - RT}{V_m}} \]  

(12)

where;

\( R \) is the gas constant \((J/K.mol)\)

\( T \) is the temperature \((K)\)

\( \Delta H_{vap} \) is the enthalpy of vaporisation \((J/mol)\)

\( E \) is the cohesive energy \((J/mol)\)

\( V_m \) is the molar volume \((cm^3/mol)\)

\( E/V_m \) is the Cohesive Energy Density (CED) \((J/cm^3)\)

Therefore, maximum lignin solubility should occur when the \( \delta \)-value of the solvent is close to that of the lignin. To apply these concepts, the \( \delta \)-value of different lignins was determined in this chapter.

It is important to know that Hildebrand solubility parameters are made based on the non-polar interactions with the absence of hydrogen bonds. Recently, Hansen developed the solubility parameters in his theory based on three specific molecular interactions; dispersive interactions \((\delta_D)\), dipole-dipole interactions \((\delta_P)\) and hydrogen bonding interactions \((\delta_H)\) (Hansen, 2000). Dispersive interactions (non-polar interactions) arise due to negatively charged electrons orbiting around a positively charged nucleus. Therefore, an electromagnetic field is created by moving negative charges which attract the atoms to one another. Another type of interaction is polar cohesive forces, which is produced by permanent dipole–dipole interactions. Hydrogen bonding is a major molecular interaction, although they are considerably weaker than covalent bonds, but they are much stronger than dipole–dipole interactions (Hansen, 2000).
Therefore, Hansen proposed these three types of interactions as the cohesive energy

\[ E = E_D + E_P + E_H \]  

(13)

Cohesive energy density is calculated by dividing the cohesive energy by the molar volume. The sum of the squares of the Hansen dispersion (D), polar (P), and hydrogen bonding (H) will be:

\[ E/V_m = E_D/V_m + E_P/V_m + E_H/V_m \]  

(14)

\[ \delta^2 = \delta^2_D + \delta^2_P + \delta^2_H \]  

(15)

5.1.5 Estimation of solubility parameters (Group contribution methods)

The \( \delta \)-value of a polymer is estimated based on the contribution of functional and atomic groups when the repeating unit (monomer) of the polymer is known (Fedors, 1974). Cohesive energy (E) and molar volume (\( V_m \)) are calculated by concerning the contributions of atomic and functional groups;

\[ E = \sum \Delta e_i \]  

(16)

and

\[ V_m = \sum \Delta v_i \]  

(17)

Where;

\( \Delta e_i \) is atomic and group contributions for the cohesive energy (E)

\( \Delta v_i \) is atomic and group contributions for the molar volume (\( V_m \))
The δ-value of lignin and lignin acetate can be calculated based on the contribution of functional and atomic groups. Energy of vaporization (E) and molar volume ($V_m$) are calculated by considering the contributions of atomic and functional groups for each phenylpropanoid unit. It is important to note that Hildebrand solubility parameters are made with the absence of hydrogen bonds. Therefore, in this study hydrogen bonding interactions from the Hansen theory were also accounted for lignin-solvent interactions.

The goal of this study is to understand the relationship between lignin chemical structure and the solubility of lignins (and their acetylated forms) isolated from different sources in various organic solvents. Based on Hildebrand theory, maximum lignin solubility should occur when the solubility parameter of the solvent is close to that of the lignin. To apply these concepts, the δ-value of different lignins and acetylated lignins were calculated based on their structural elements and functional groups.

5.2 Experimental

5.2.1 Materials

Isolated lignin from bioethanol biorefinery residue, kraft black liquor and two commercial lignins were used in this study. L1 and L2 were isolated from the bioethanol biorefinery residue and kraft black liquor, respectively. Two commercial lignins; L3 (Indulin AT, softwood kraft pine lignin) and L4 (Protobind 2000, non-wood soda lignin) were supplied by Westvaco Co. and ALM Private Limited, respectively.

Eleven organic solvents and water were used in this experiment. Solvents were purchased as follows: diethyl ether (Sigma); chloroform (Sigma); acetone (BDH); ethyl acetate (Fisher); dichloromethane (DCM) (Caledon); dioxane (Caledon); dimethyl sulfoxide (DMSO) (Caledon); ethanol (Caledon); methanol (Caledon), pyridine (Caledon), tetrahydrofuran (THF) (Caledon).
5.2.2 Acetylation of lignin

The method used for acetylation of lignin samples follows that use by Olarte (2011) (Olarte, 2011). 1.0 g lignin was mixed with 40 ml of pyridine-acetic anhydride (50-50%) solution. The mixture was allowed to react for 24 hours while mixing. The solids were re-precipitated with 150 ml of hydrochloric acid solution (pH = 1.0) and collected using a vacuum filtration technique. The solids were washed with some HCl solution and then with deionized water. The collected solids were dried at 40°C for overnight and stored in vials for further analysis.

5.2.3 Solubility determination of lignin in different organic solvents

The solubility of lignin in organic solvents was determined based on the method described by Cybulska et al., 2012 (Cybulska et al., 2012) with minor modification. 100 mg (oven dried) lignin or lignin acetate was dissolved in 10 mL of organic solvent at room temperature. Samples were sonicated for 10 minutes in water bath sonicator. The insoluble fraction (if present) was filtered by using medium size (10-15µm pore size) of filter crucible. Then samples were dried at 50°C for 4h, and weighed. The soluble fraction of 100 mg lignin or lignin acetate in 10 mL organic solvent was calculated by subtracting the insoluble fraction from the initial weight.

5.2.4 Determination of hydroxyl content using $^{31}$PNMR

Total aliphatic hydroxyl, phenolic hydroxyl and carboxyl groups of lignin samples were determined using the method developed by Granata and Argyropoulos (Granata and Argyropoulos, 1995). A solvent solution of CDCl$_3$ (1.6/1, v/v) and pyridine was prepared for dissolving lignin and other reagents. Phosphitylation reagent was 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) for lignin phosphitylating. The internal standard solution (cholesterol, 85 mg/mL) and the relaxation reagent solution (chromium(III) acetylacetonate, 5.6 mg/mL) were prepared with the same solvent solution. 40.0 mg of dried lignin was dissolved in 500 µL of the solvent solution in a sealed vial; this was followed by the addition of 100 µL of the internal standard and 50
µL of relaxation solution. Then, 100 µL of phosphitylation reagent was added, and the vial was shaken to ensure a homogeneous mixture. After derivatization, the resulting solution was transferred to a 3-mm tube, and the $^{31}$P-NMR spectrum was recorded with a Varian Unity Plus 600 MHz spectrometer.

5.2.5 Molecular weight determination using HPSEC

Molecular mass distributions of soluble and insoluble parts of four lignin samples in EA were determined by the method described by Gonzalez 2000 (González et al., 2000). In this method High Performance Size Exclusion Chromatography (HPSEC) was performed to determine the molecular mass distribution of lignin sample in alkaline solution. HPSEC method was carried out with a DIONEX DX600 chromatograph equipped with an UV detector and a PSS MCX column (1000 Å, 300 x 8 mm). The UV detection was carried out at the wavelength of 280 nm at room temperature (25 °C). UV detector was adjusted at 280 nm due to the maximum UV absorption of the lignins. This wavelength was used to estimate for molar concentration of the aromatic rings. The injection was 25 µL. Eluent (0.1 M NaOH solution) was prepared with deionized water (Millipore water from a purification system). Sodium poly(styrene sulfonate) which is known to exhibit a similar behavior with lignin was used for calibration of the column. Sodium poly(styrene sulfonates) standards (6520, 4230, 1830 and 1100 daltons) were purchased from Polymer Standard Services - USA Incorporation. Calibration curve was prepared by adding 10 mg of each polystyrene standard in 10 mL water. Each lignin sample was prepared by dissolving 10 mg of the dry lignin in 100 mL of 0.1 M sodium hydroxide solution. The stationary phase of this column is sulfonated styrene-divinylbenzene copolymer-network which is appropriate for carrying out HPSEC experiments over the whole 7-13 pH range. The number and weight average molecular weights were calculated based on the ASTM D5296 –11.
5.3 Results and discussion

5.3.1 Computing $\delta$-value of lignin and lignin acetate based on the expanded C9 formula

Typical structures for phenylpropane units (G, S and H) are shown in Figure 22. Table 20 shows data concerning the atomic and functional group contributions to $e_i$ and $v_i$ which exist in each lignin unit.

![Figure 22. Typical repeating units of lignin (Quesada-Medina et al., 2010)](image)

Table 20. Values of $\Delta e_i$ and $\Delta v_i$ for atoms and groups in lignin (Fedors, 1974; Ni and Hu, 1995)

<table>
<thead>
<tr>
<th>Atom or group</th>
<th>$\Delta e_i$ (cal/mol)</th>
<th>$\Delta v_i$ (cm$^3$/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>7120</td>
<td>10.0</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>1180</td>
<td>16.1</td>
</tr>
<tr>
<td>C=</td>
<td>1030</td>
<td>-5.5</td>
</tr>
<tr>
<td>CH</td>
<td>820</td>
<td>-1.0</td>
</tr>
<tr>
<td>Phenyl, S</td>
<td>7630</td>
<td>14.4</td>
</tr>
<tr>
<td>Phenyl, G</td>
<td>7630</td>
<td>33.4</td>
</tr>
<tr>
<td>Phenyl, H</td>
<td>7630</td>
<td>52.4</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>1925</td>
<td>37.3</td>
</tr>
<tr>
<td>O</td>
<td>800</td>
<td>3.8</td>
</tr>
<tr>
<td>CH$_3$COO</td>
<td>5550</td>
<td>50.5</td>
</tr>
</tbody>
</table>
Based on the number of double bonds and number of the atom groups in C9 formula and the ratio of G/S/H in each unit, we calculated the $\Delta e_i$ and $\Delta v_i$ for each lignin sample (Table 21). Solubility parameter of each lignin is calculated by the square root of the sum of $\Delta e_i$ divided by sum of $\Delta v_i$ in equation (12). The solubility parameters for L1, L2, L3 and L4 were calculated as 12.87, 13.09, 13.42 and 12.92 (cal/cm$^3$)$^{1/2}$, respectively. These values are slightly lower than the value reported for other type of lignins in previous reports; ALCELL lignin (13.7 (cal/cm$^3$)$^{1/2}$) (Ni and Hu, 1995), Bagasse lignin (14.0 (cal/cm$^3$)$^{1/2}$) (Wang et al., 2011), and hydrolyzed almond shell lignin (14.6 (cal/cm$^3$)$^{1/2}$) (Quesada-Medina et al., 2010).
Table 21. Calculated $\Delta e_i$ and $\Delta v_i$ for each lignin based on the number of the functional group and the ratio of G/S/H (*$\Delta v_i$ is the correction factor for divergence in the v value (Ni and Hu, 1995)).

<table>
<thead>
<tr>
<th>Atom or group</th>
<th>L1</th>
<th></th>
<th></th>
<th>L2</th>
<th></th>
<th></th>
<th>L3</th>
<th></th>
<th></th>
<th>L4</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Atom or group</td>
<td># of atom or group</td>
<td>$\Delta e_i$</td>
<td>$\Delta v_i$</td>
<td># of atom or group</td>
<td>$\Delta e_i$</td>
<td>$\Delta v_i$</td>
<td># of atom or group</td>
<td>$\Delta e_i$</td>
<td>$\Delta v_i$</td>
<td># of atom or group</td>
<td>$\Delta e_i$</td>
<td>$\Delta v_i$</td>
</tr>
<tr>
<td>OH</td>
<td>0.85</td>
<td>6052</td>
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<td>0.59</td>
<td>4201</td>
<td>5.9</td>
<td>0.92</td>
<td>6550</td>
<td>9.2</td>
<td>0.71</td>
<td>5055</td>
<td>7.1</td>
</tr>
<tr>
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<td>1180</td>
<td>16.1</td>
<td>1.00</td>
<td>1180</td>
<td>16.1</td>
<td>1.00</td>
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<td>16.1</td>
<td>1.00</td>
<td>1180</td>
<td>16.1</td>
</tr>
<tr>
<td>C=</td>
<td>1.50</td>
<td>1541</td>
<td>-8.2</td>
<td>1.35</td>
<td>1390</td>
<td>-7.4</td>
<td>1.23</td>
<td>1265</td>
<td>-6.8</td>
<td>1.17</td>
<td>1207</td>
<td>-6.4</td>
</tr>
<tr>
<td>CH</td>
<td>1.00</td>
<td>820</td>
<td>-1.0</td>
<td>1.00</td>
<td>820</td>
<td>-1.0</td>
<td>1.00</td>
<td>820</td>
<td>-1.0</td>
<td>1.00</td>
<td>820</td>
<td>-1.0</td>
</tr>
<tr>
<td>Phenyl, S</td>
<td>0.26</td>
<td>1984</td>
<td>3.7</td>
<td>0.68</td>
<td>5188</td>
<td>9.8</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0.51</td>
<td>3891</td>
<td>7.3</td>
</tr>
<tr>
<td>Phenyl, G</td>
<td>0.31</td>
<td>2365</td>
<td>10.4</td>
<td>0.31</td>
<td>2365</td>
<td>10.4</td>
<td>0.95</td>
<td>7249</td>
<td>31.7</td>
<td>0.40</td>
<td>3052</td>
<td>13.4</td>
</tr>
<tr>
<td>Phenyl, H</td>
<td>0.44</td>
<td>3357</td>
<td>23.1</td>
<td>0.01</td>
<td>76</td>
<td>0.5</td>
<td>0.05</td>
<td>382</td>
<td>2.6</td>
<td>0.09</td>
<td>687</td>
<td>4.7</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>1.37</td>
<td>2637</td>
<td>51.1</td>
<td>1.45</td>
<td>2791</td>
<td>54.1</td>
<td>1.02</td>
<td>1964</td>
<td>38.0</td>
<td>1.41</td>
<td>2714</td>
<td>52.6</td>
</tr>
<tr>
<td>O</td>
<td>1.21</td>
<td>968</td>
<td>4.6</td>
<td>1.31</td>
<td>1048</td>
<td>5.0</td>
<td>0.27</td>
<td>216</td>
<td>1.0</td>
<td>0.34</td>
<td>272</td>
<td>1.3</td>
</tr>
<tr>
<td>CH$_3$COO</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\Delta v_i$*</td>
<td>1.00</td>
<td>0</td>
<td>18.0</td>
<td>1.00</td>
<td>0</td>
<td>18.0</td>
<td>1.00</td>
<td>0</td>
<td>18.0</td>
<td>1.00</td>
<td>0</td>
<td>18.0</td>
</tr>
<tr>
<td>Sum</td>
<td>20905</td>
<td>126.2</td>
<td>19060</td>
<td>111.3</td>
<td>19624</td>
<td>109.0</td>
<td>18879</td>
<td>113.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility Parameter</td>
<td>12.87</td>
<td>13.09</td>
<td>13.42</td>
<td>12.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The solubility parameter of lignin acetate was calculated in a similar way as calculated for lignin by replacing acetate groups with total hydroxyl groups in each unit (Table 22). We assumed that all hydroxyl groups were substituted with acetyl groups. The solubility parameter for ACL1, ACL2, ACL3 and ACL4 was obtained 10.97, 11.32, 11.03 and 11.04 (cal/cm$^3$)$^{1/2}$, respectively. The calculated solubility parameter of lignin acetate is lower than its original lignin, because the cohesive energy for hydroxyl group is 26.7 (cal/cm$^3$)$^{1/2}$, while for acetyl group is 10.5 (cal/cm$^3$)$^{1/2}$. Therefore, the difference between solubility parameters between lignin and lignin acetate depends on the number of hydroxyl groups in lignin molecule.
Table 22. Calculated $\Delta e_i$ and $\Delta v_i$ for each lignin acetate based on the number of the functional group and the ratio of G/S/H (*$\Delta v_i$ is the correction factor for divergence in the $v$ value)

| Atom or group | ACL1 | | ACL2 | | ACL3 | | ACL4 | |
|---------------|------|------|------|------|------|------|------|
|               | # of atom or group | $\Delta e_i$ | $\Delta v_i$ | # of atom or group | $\Delta e_i$ | $\Delta v_i$ | # of atom or group | $\Delta e_i$ | $\Delta v_i$ |
| OH            | 0.00 | 0    | 0.0  | 0.00 | 0    | 0.0  | 0.00 | 0    | 0.0  |
| CH$_2$        | 1.00 | 1180 | 16.1 | 1.00 | 1180 | 16.1 | 1.00 | 1180 | 16.1 |
| C=            | 1.50 | 1541 | -8.2 | 1.35 | 1390 | -7.4 | 1.23 | 1265 | -6.8 |
| CH            | 1.00 | 820  | -1.0 | 1.00 | 820  | -1.0 | 1.00 | 820  | -1.0 |
| Phenyl, S     | 0.26 | 1984 | 3.7  | 0.68 | 5188 | 9.8  | 0.00 | 0    | 0.0  |
| Phenyl, G     | 0.31 | 2365 | 10.4 | 0.31 | 2365 | 10.4 | 0.95 | 7249 | 31.7 |
| Phenyl, H     | 0.44 | 3357 | 23.1 | 0.01 | 76   | 0.5  | 0.05 | 382  | 2.6  |
| OCH$_3$       | 1.37 | 2637 | 51.1 | 1.45 | 2791 | 54.1 | 1.02 | 1964 | 38.0 |
| O             | 1.21 | 968  | 4.6  | 1.31 | 1048 | 5.0  | 0.27 | 216  | 1.0  |
| CH$_3$COO     | 1.31 | 7271 | 66.2 | 1.45 | 8048 | 73.2 | 1.59 | 8825 | 80.3 |
| $\Delta v_i*$ | 1.00 | 0    | 18.0 | 1.00 | 0    | 18.0 | 1.00 | 0    | 18.0 |
| Sum           | 22124 | 183.9 | 22907 | 178.6 | 21899 | 180.1 | 22038 | 180.7 |
| Solubility Parameter | 10.97 | 11.32 | 11.03 | 11.04 |
Based on the Hildebrand theory, lignin (or lignin acetate) shows maximum solubility when the δ-value of the solvent is close to its own. Conversely, the solubility of the lignin (or lignin acetate) in the solvent is lower when the difference between the two δ-values shows greater value. Therefore, based on the solubility parameters of organic solvents the degree of lignin (or lignin acetate) solubility as predicted by the solubility parameter theory, should agree with the experimental results.

The δ-value from Hildebrand theory and δ_h-value from Hansen theory of each solvent was listed in Table 23 (Burke, 1984). The solubility of lignin and lignin acetate will be discussed based on the Hildebrand theory in the following section. Hydrogen-bonding parameter in Hansen theory would be useful in some explanation. The δ_h-value of organic solvents such as ethanol or methanol is much higher than other organic solvents due to hydrogen bonding. It is important to know that lignin with higher hydroxyl content may dissolve in such solvents with higher δ_h-value.

Table 23. δ–value (from Hildebrand theory) and δ_h-value (from Hansen theory) of organic solvents and water (Hansen, 2000; Hildebrand and Scott, 1950)

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent</th>
<th>δ</th>
<th>δ_h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diethyl Ether</td>
<td>7.6</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl Acetate (EA)</td>
<td>9.1</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>9.2</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>Tetrahydrofuran (THF)</td>
<td>9.5</td>
<td>3.9</td>
</tr>
<tr>
<td>5</td>
<td>Acetone (ACE)</td>
<td>9.8</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>Dichloromethane (DCM)</td>
<td>9.9</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>Dioxane</td>
<td>10.0</td>
<td>4.4</td>
</tr>
<tr>
<td>8</td>
<td>Pyridine</td>
<td>10.6</td>
<td>2.9</td>
</tr>
<tr>
<td>9</td>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>12.9</td>
<td>5.0</td>
</tr>
<tr>
<td>10</td>
<td>Ethanol</td>
<td>12.9</td>
<td>9.5</td>
</tr>
<tr>
<td>11</td>
<td>Methanol</td>
<td>14.3</td>
<td>10.9</td>
</tr>
<tr>
<td>12</td>
<td>Water</td>
<td>23.5</td>
<td>20.6</td>
</tr>
</tbody>
</table>
5.3.2 Solubility of lignins from different sources in organic solvents

Figure 23 shows the solubilized fraction of 100 mg lignin in 10mL of different organic solvents together with the Hildebrand solubility parameter (δ) of organic solvents. The solubility parameter for lignin samples was obtained in a range of 13.0 (cal/cm$^3$)$^{1/2}$ to 13.5 (cal/cm$^3$)$^{1/2}$.

100 mg of lignin samples (L1, L2, L3 and L4) were completely soluble in 10 mL of DMSO and pyridine, while partially soluble in other 9 organic solvents and water. The δ-values of DMSO and pyridine are 12.9 and 10.6 (cal/cm$^3$)$^{1/2}$, respectively. Based on the Hildebrand theory, it is expected that when the δ-values of lignin and the solvent were the same, the ability to dissolve lignin increased. Therefore, DMSO with similar solubility parameter with lignin, is one of the best solvents for lignin. Pyridine is also a good lignin solvent even having a smaller δ-value than lignin. This behavior can be explained by an acid–base interaction between pyridine and the phenolic groups in lignin, resulting in the high solubility of lignin in pyridine (Shukry et al., 2008). It is important to note that the chemical interactions such as acid-base interaction are not considered in Hildebrand theory.

Solubility parameter of ethanol (δ =12.9) is very similar to the solubility parameter of lignin samples, but the solubility of lignin in ethanol is lower than the expected value. Low solubility of lignin in ethanol can be explained based on the Hansen theory where the δ$_h$ value (9.5) of ethanol is very high. Shukry et al., reported that ethanol was not efficient for dissolving acetosolv lignins due to a high value of δ$_h$ (Shukry et al., 2008). Although the solubility parameter of lignin is more close to the solubility parameter of ethanol than methanol, it seems methanol is a better solvent for lignin. This is due to the smaller molar volume of methanol than ethanol. The dissolution rates are strongly dependent on molar volume of methanol and ethanol because penetration rate increases with decreasing solvent size (Papanu et al., 1990). The molar volume of the solvents was not considered in the Hildebrand theory.

In addition, note that the soluble fraction of 100 mg L3 in 10 mL ethanol is about 10mg, while in 10 mL methanol is about 60 mg. The reason for this big difference may be
explained by a number of aliphatic hydroxyl groups in L3. The results from $^{31}$PNMR analysis and expanded C9-formula showed that the number of aliphatic hydroxyl groups in L3 is more than the other lignin samples (see Table 11 and Table 14). Higher number of hydroxyl group increases the hydrogen bonding that can increase the solubility of lignin in hydroxylated solvents. Horvath stated that the solubility of lignin is greater with hydroxylated solvents, e.g., methanol and ethanol than nonpolar solvents like diethyl ether (Horvath, 2005). In addition, based on the Hansen theory, the solubility of L3 in methanol is more than ethanol because the $\delta_h$ value for methanol is greater than for ethanol.

As it was expected, solubility of all lignin samples was very low in water due to big differences in solubility parameters of lignin and water. However, a direct correlation was observed between the number of OH groups in C9 formula and the solubility of lignins in water. The solubility of lignin in water increases by increasing the number of OH groups in C9-formula.

Dioxane, DCM and acetone have similar $\delta$-value which is about 10 (cal/cm$^3$)$^{1/2}$. It was reported that kraft lignin (commercial L3) exhibits maximum solubility in solvents having a Hildebrand’s solubility parameter 10.0 – 11.0 (cal/cm$^3$)$^{1/2}$ (Rahman et al., 2013), while our results showed that L3 was moderately soluble in dioxane and slightly soluble in DCM and acetone. The reason for higher solubility of lignin in dioxane might be due to formation of hydrogen bonding between lignin and solvent ($\delta_h$-value of dioxane is greater than acetone and DCM). In addition, L3 with high number of aliphatic hydroxyl groups showed more solubility in dioxane than other organic solvents.

The solubility parameter of THF, chloroform and EA is in the order of 9.5 > 9.2 > 9.1. THF exhibit better solvent than chloroform and EA for lignins due to its closer solubility parameter to the solubility parameters of lignins. However, the results showed that the solubility of lignin in EA is higher than the expected value based on the Hildebrand theory.

Rahman and co-workers (Rahman et al., 2013) reported that 10 mg L3 (commercial lignin) is completely soluble in 1 mL DMSO, while it is partially soluble in THF and
chloroform. We have also found that 100mg L3 is almost soluble in 10 mL DMSO (96 mg/10 mL), but it is slightly soluble in THF (8 mg/10 mL) and it is almost not soluble in chloroform. Cybulska et al., (2012) reported the solubility of different type of organosolv lignins (i.e. prairie cordgrass, switchgrass and corn stover lignins) in organic solvents. They found the highest solubility of lignins in methanol and dioxane and non-significantly soluble in ethyl acetate (Cybulska et al., 2012).

Solubility of lignin in diethyl ether is very low due to significant difference between δ-value of all lignins and the solvent, and also due to very low δ_H-value of the solvent.

![Figure 23. Solubility of 100 mg lignin in 10 mL of different organic solvents](image)
5.3.3 The effect of lignin molecular weight on the solubility

There are several aspects in polymer dissolution, one of which is the polymer molecular weight that affect its dissolution. Figure 24 shows the relationship between the solubility of four lignin samples and organic solvents. It was found that the solubility of lignins with uniform size distribution was increased with decreasing molecular mass of the lignin. Solubility of L1 in organic solvents was higher than the expected values due to its biomodal molar mass distribution.

L2 with lowest molecular weight is dissolved in organic solvents more than other lignins. It was reported that the lignins with lower molecular weight are more soluble in the most common organic solvents (Alriols et al., 2009; Horvath, 2005). As expected, the solubility of L3 was lower than L2 and L4 lignins due to its higher molecular weight than L2 and L4 lignins.

The effects of molecular weight on the dissolution rates of thin poly(methyl methacrylate), (PMMA) films showed a non-linear behavior when the log dissolution rate was plotted against the log Mn (Cooper et al., 1985). Manjkow et al. (Manjkow et al., 1987) found that dissolution of polymer depends on polymer molecular weight and polydispersity.

The dissolution of polymer is controlled by chain disentanglement, which is a function of the molecular weight (Parsonage et al., 1987). Polymers with smaller molecular weights yield higher degree of disentanglement. As a result, lower molecular weights have a higher degree of swelling when dissolution occurs.
Figure 24. The relationship between solubility and weight average molar mass of lignin samples (L1-L4)

5.3.4 Solubility of acetylated lignins in organic solvents

The solubility of acetylated lignins in organic solvents is demonstrated in Figure 25. Acetylation of lignin is a technique that is used to increase the solubility of lignin in organic solvents such as THF and DMSO (Olarte, 2011). Our study showed that 100 mg of all four acetylated lignins are completely soluble in 10 mL of ethyl acetate, chloroform, THF, dichloromethane, acetone and pyridine (δ-value from 9.1 to 10.6) due to the similar solubility parameters of acetylated lignins and solvents. Although, the solubility parameter of DMSO is much greater than acetylated lignins, but it is still a good solvent for all acetylated lignins. DMSO has very high dipole moment and it is a good solvent for large lipophilic compounds which have some type of dipole moment in the structure. DMSO with large dielectric constant is energetically able to interact with large hydrophobic molecules that contains functional groups possessing a dipole moment (Borchardt et al., 2005). Therefore, solubility of acetylated lignin in DMSO is
high because the number of dipole carbonyl groups is significantly increased after acetylation. Dipole moment is the factor that was not considered in Hildebrand theory, but it was explained by Hansen theory.

The solubility parameter of diethyl ether is much lower than acetylated lignins, therefore, diethyl ether is considered as a poor solvent for lignin acetate. Very low solubility of acetylated lignins in methanol, ethanol and water can be explained by the significant differences between δ-value of the solvent and the acetylated lignins.

Figure 25. Solubility of 100 mg of acetylated lignin in 10 ml of different organic solvents
5.3.5 Solubility of lignin in ethyl acetate

For the technique of microspheres fabrication by solvent evaporation, a suitable solvent should: (i) be able to dissolve the polymer, (ii) have low boiling point and high volatility (iii) be poorly soluble in water and (iv) have low toxicity (Li et al., 2008).

Dichloromethane (DCM) is the most common solvent for microsphere preparation because of its low boiling point, high immiscibility in water and high volatility. However, DCM is considered as carcinogenic according to environmental protection agency (EPA), and many efforts have been done to find solvents with less toxicity (Li et al., 2008). In addition, lignin should be modified to lignin acetate in the first step of the process to become soluble in DCM.

Ethyl acetate (EA) as a less toxic organic solvent has the potential to be substituted with DCM. But miscibility of EA in water is the limitation (Freytag et al., 2000). Some methods have been suggested in the literature to overcome this problem caused by the miscibility of solvent with water (Bahl and Sah, 2000).

Therefore, more details were revealed on the physico-chemical properties of soluble part of lignin samples in EA. The hydroxyl content and the molar mass distribution of soluble and insoluble lignin samples in EA were analyzed by using $^{31}$PNMR and SEC.

The objective of this study was to determine the relationship between the molar mass distribution and number of OH groups and the solubility of lignin in ethyl acetate.

5.3.6 Hydroxyl content of soluble part of lignin in ethyl acetate

The aliphatic and phenolic hydroxyl content was determined for the soluble part of lignin in EA by using $^{31}$PNMR (Figure 26). The different hydroxyl groups in lignin samples were obtained by integration of each spectral region. The signals in the range of 149.2 ppm to 146.0 ppm are associated with aliphatic hydroxyls groups. The phenolic hydroxyl region is in the range of 144.3 ppm to 137.2 ppm. Signals in the range of 143.1-142.4 ppm (144.3-140.5 ppm for softwood kraft lignin (Monteil-Rivera et al., 2013)), 140.0-138.8 ppm, 138.2-137.2 ppm and 135.6–133.7 ppm are attributed to syringyl, guaiacyl,
p-hydroxyphenyl phenolic hydroxyls and carboxylic acid units, respectively (Cateto et al., 2008; Zhang et al., 2013a).

Figure 26. $^{31}$PNMR spectrum of lignin samples and their soluble part in EA

Figure 27 shows the number of phenolic and aliphatic hydroxyl of lignin samples and the soluble part of lignin samples in EA. It is clear that the aliphatic hydroxyl content was decreased in soluble part of lignin samples. Therefore, it seems that the part of lignin macromolecules with less hydroxyl group is more soluble in EA.
Figure 27. Phenolic hydroxyl and aliphatic hydroxyl content of lignin and soluble part of lignin in EA
5.3.7 Molecular weight of soluble and insoluble part of lignin in ethyl acetate

The calibration curves of the standards were obtained for determination of the molecular weight of soluble and insoluble part of lignin in EA (Figure 28).

![ Calibration curve of the PSS standards for molecular weight determination of a) insoluble part of lignins in EA and b) soluble part of lignins in EA ]

Figure 28. Calibration curve of the PSS standards for molecular weight determination of a) insoluble part of lignins in EA and b) soluble part of lignins in EA

Figure 29 shows the molar mass distribution of each original lignin sample and also soluble and insoluble part of lignin samples in ethyl acetate. The molar mass distribution of insoluble part of lignin samples in EA showed higher molar masses while molar mass distribution of soluble part of lignin in EA was shifted to lower molar mass values. It is clear that the portion of lignin with lower molar mass was dissolved in ethyl acetate, and the higher molar mass remained insoluble. It was found that the highest molar mass of all soluble lignin samples in EA was about 10 KDa. In other words, EA is able to dissolve lignin macromolecules with the molecular weight less than 10 KDa.
It is interesting to compare the molecular weight distribution of soluble part of different lignins in EA (Figure 30). Although the solubility of each lignin is different in EA (see Figure 23), it seems that the size distribution for all soluble part of the lignin samples were the same.
The Mw, Mn, Mp and PD of soluble and insoluble part of lignin samples in EA were summarized in Table 24. The data showed that Mw, Mn, Mp and DP of soluble part of all lignin samples in EA were lower than in the original lignin, while the insoluble lignin in EA showed greater values than original lignin. Figure 31 shows the differences between the Mw of four lignin samples.

The solubility of four lignin samples in 10 mL EA was found to be 23.7, 60.6, 8.8 and 42.7 mg for L1, L2, L3 and L4, respectively (Figure 23). About 75% of L1 was not soluble in EA due to very high Mw and bimodal molar mass distribution. It is important to note that the soluble part of L1 in EA contains the lowest molecular weight among all four lignin samples. L2 with the solubility of about 60 mg/10mL in EA was the most soluble lignin in this study.
Table 24. The number average (Mn), weight average (Mw), peak average (Mp) molecular weights and polydispersity (PD) of soluble and insoluble part of lignin in ethyl acetate (EA)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mn</th>
<th>Mw</th>
<th>PD</th>
<th>Mp1</th>
<th>Mp2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1093</td>
<td>13488</td>
<td>12.3</td>
<td>1727</td>
<td>74680</td>
</tr>
<tr>
<td>Insoluble part of L1 in EA</td>
<td>1804</td>
<td>18933</td>
<td>10.5</td>
<td>4258</td>
<td>57306</td>
</tr>
<tr>
<td>Soluble part of L1 in EA</td>
<td>575</td>
<td>1316</td>
<td>2.3</td>
<td>821</td>
<td>x</td>
</tr>
<tr>
<td>L2</td>
<td>866</td>
<td>2565</td>
<td>3.0</td>
<td>1848</td>
<td>x</td>
</tr>
<tr>
<td>Insoluble part of L2 in EA</td>
<td>1338</td>
<td>5213</td>
<td>3.9</td>
<td>3299</td>
<td>x</td>
</tr>
<tr>
<td>Soluble part of L2 in EA</td>
<td>687</td>
<td>2020</td>
<td>2.9</td>
<td>1172</td>
<td>x</td>
</tr>
<tr>
<td>L3</td>
<td>1191</td>
<td>6096</td>
<td>5.1</td>
<td>2447</td>
<td>x</td>
</tr>
<tr>
<td>Insoluble part of L3 in EA</td>
<td>1253</td>
<td>9498</td>
<td>7.6</td>
<td>3235</td>
<td>x</td>
</tr>
<tr>
<td>Soluble part of L3 in EA</td>
<td>566</td>
<td>1430</td>
<td>2.5</td>
<td>847</td>
<td>x</td>
</tr>
<tr>
<td>L4</td>
<td>1084</td>
<td>5008</td>
<td>4.6</td>
<td>2593</td>
<td>x</td>
</tr>
<tr>
<td>Insoluble part of L4 in EA</td>
<td>1254</td>
<td>6540</td>
<td>5.2</td>
<td>3465</td>
<td>x</td>
</tr>
<tr>
<td>Soluble part of L4 in EA</td>
<td>934</td>
<td>3023</td>
<td>3.2</td>
<td>874</td>
<td>x</td>
</tr>
</tbody>
</table>
5.4 Conclusions

This study showed that the solubility of different lignin samples (and lignin acetates) in organic solvents was not completely predictable by using Hildebrand solubility parameters. However, it was found that the solubility parameter of lignin acetates was closer to organic solvents than unmodified lignins. Acetylated lignins are completely soluble in ethyl acetate, chloroform, THF, acetone, DCM, dioxane, pyridine and DMSO. Unmodified lignins exhibit maximum solubility only in pyridine and DMSO. Hardwood kraft lignin (L2) and soda non-wood lignin (L4) are moderately soluble in ethyl acetate, THF, acetone and dioxane. Solubility of lignin in organic solvents depends on the molecular weight of lignin and the number of hydroxyl groups in lignin units. Lignins only with certain molecular weight (less than 10KDa) are soluble in ethyl acetate. The number of hydroxyl groups in soluble part of lignin in EA was lower than the unmodified lignin.
CHAPTER 6 Preparation and characterization of lignin microspheres

First part of this chapter was published with the title “Effect of Preparation Parameters on the Formation of Lignin Acetate Microspheres” in International Journal of Engineering and Innovative Technology, 2015, Volume 4, issue 8, 102-113. Authors: Sameni, J., Krigstin, S., Sain, M.,

6.1 Introduction

Lignin is produced in large quantities as a by-product in the pulp industries and biorefineries (Doherty et al., 2011). Although, there are several methods to isolate lignin, because of its varying molecular weight, functional groups and unknown molecular structure it becomes less applicable in many areas (Lora and Glasser, 2002). However, even with these drawbacks, the interest for developing lignin-based products is growing as a result of an increase in the demand for advanced sustainable products (Larry Hughes, 2014; Mousavioun and Doherty, 2010). Preparation of lignin nano- or microspheres could be useful in many applications such as agricultural actives controlled release (Asrar and Ding, 2010; Chowdhury, 2014; Fernandez-Perez et al., 2011), food industry fat mimetics (Stewart et al., 2014), filler in composites (Jiang et al., 2013) and nano-sized coatings (Popa et al., 2011).

Lignin microspheres can be synthesized through emulsion solvent evaporation technique; if lignin is completely dissolved in a suitable organic solvent. However, the solubility of lignin is very low in many organic solvents due to presence of hydrophilic moiety in the lignin molecule (Shukry et al., 2008). Therefore, two strategies were proposed for synthesis of lignin microspheres: 1. modifying the lignin to lignin acetate to improve the solubility of the lignin in organic solvents and 2. using the soluble part of the lignin in the organic solvent. In both cases, the organic phase contains solubilized lignin, but with two variables: A. lignin and lignin acetate and B. lignin from different sources. The difference between these lignins is the molecular weight and number of the
hydroxyl groups. Therefore, the effects of the molecular weight and the number of hydroxyl groups of lignins on the microparticles formation will be discussed in this section.

Among all organic solvents, dichloromethane and chloroform (chlorinated solvents that threaten human safety and have environmental concerns) have been widely used as solvents in the emulsion solvent evaporation technique. In order to reduce the use of these toxic solvents, many attempts have been made to prepare the polymer microspheres using a solvent with lower toxicity, such as ethyl acetate, as the dispersing solvent. The effect of ethyl acetate as a dispersing solvent was studied in the production of different polymers such as PLGA microspheres (Soppimath and Aminabhavi, 2002). Ethyl acetate has not been used in producing lignin microspheres and, hence, in this thesis it was attempted to prepare lignin microspheres by different lignin samples using ethyl acetate as dispersing solvent.

6.1.1 Methods for synthesis of micro/nanoparticles

Synthetic polymeric micro/nanoparticles such as polystyrene, polyalkyl(meth)acrylates, polyesters and polyurethanes have been designed for various applications. Also biodegradable polymers such as poly(lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA) have been investigated to formulate micro/nanoparticle-based drug carriers (Freiberg and Zhu, 2004). Vasir et al., (2003) provided an interesting review concerning bio-adhesive microspheres for controlled drug delivery system (Vasir et al., 2003).

Biodegradable polymers, such as PLA and PLGA are mainly fabricated into micro/nanoparticles by physical methods, including emulsification (Arshady, 1991), spray-drying (Baras et al., 2000), precipitation (Young et al., 1999), emulsion solvent evaporation (Gurny et al. 1996), salting-out procedure (Ibrahim et al., 1992), and nanoprecipitation procedure (Fessi et al., 1989). All these methods involve dissolving polymers into solution, disintegrating the solution into droplets, and subsequently removing the solvent to obtain solid particles. Emulsion solvent evaporation has been the most commonly used method for preparation of polymer microspheres (Conti et al.,
1991). The technique of microencapsulation by emulsion solvent evaporation is widely applied in pharmaceutical industries to obtain the controlled release of drugs (Li et al., 2008).

6.1.2 Emulsion solvent evaporation technique

The emulsion solvent evaporation technique involves three major steps: droplet formation, solvent removal, and drying. Oil droplets (dissolved polymer in an organic solvent) are formed in the aqueous continuous phase when the organic phase is intermixed with the aqueous phase (Asrar and Ding, 2010). After the formation of the emulsion in the first step, the liquid droplets of the organic phase are transformed into solid spherical nano/micro particles by removing the organic solvent from the emulsion (Freiberg and Zhu, 2004; Ravi et al., 2008; Silva et al., 2005). Accompanied by the solvent evaporation, the drops of the dispersed phase become rich in polymer due to solvent removal and they begin to solidify (Li et al., 2008).

6.1.3 Emulsion solvent evaporation technique for synthesis of lignin microspheres

The lignin-based microparticles can be produced by emulsion solvent evaporation technique that contains lignin in a volatile organic solvent. It is important to note that the organic solvent plays a crucial role on the synthesis of lignin microspheres. In the first step, solubilized lignin in organic solvent is intermixed with an aqueous solution containing emulsifier to form an emulsion. After the emulsion has been formed, the organic solvent is removed, thereby producing uniform spherical lignin microparticles.

Figure 32 shows the process for synthesis of lignin microspheres. First the two phases were placed in the mixer (A), then the system was disturbed to make an emulsified lignin-containing organic solution with an aqueous surfactant solution to make an oil-in-water emulsion (B). Emulsion droplets are trapped in the micelles when the agitation is stopped, and solvent begins to evaporate from the system (C). Lignin microparticles are formed and stabilized by complete evaporation (Solidification) (D).
6.1.4 Solvent choice

For synthesis of microspheres through emulsion solvent evaporation technique, the organic solvent should (i) have low boiling point and high volatility (ii), be able to dissolve the polymer (iii), be poorly soluble in water and (iv) have low toxicity (Li et al., 2008; Soppimath and Aminabhavi, 2002). For instance, dichloromethane, chloroform, THF, acetone and ethyl acetate are classified in this group of solvents because these solvents have high volatility and capacity to dissolve most polymers and their boiling point is lower than the normal boiling point of water. Among these organic solvents, DCM is the most common solvent for synthesis of microsphere due of its low boiling point, high immiscibility and high volatility. Shorter duration for microspheres fabrication occurs due to its high evaporation rate. However, DCM is considered as carcinogenic according to environmental protection agency (EPA), and many efforts have been made to find solvents with less toxicity (Li et al., 2008).

Ethyl acetate as a less toxic organic solvent and has great potential to be a substitute for DCM. But miscibility of ethyl acetate in water is the limitation if the dispersed phase
is directly introduced into the continuous phase. The polymer is precipitated into fiber-like agglomerates due to sudden extraction of a large portion of ethyl acetate from the dispersed phase into the continuous phase (Freytag et al., 2000). Two methods have been suggested in literatures to overcome this problem caused by the miscibility of solvent with water. Either the aqueous solution is pre-saturated with solvent (Bahl and Sah, 2000), or the dispersed phase first is emulsified in a little quantity of aqueous solution, then the solution is agitated and poured into a large quantity of aqueous solution (Freytag et al., 2000).

6.1.5 Surfactant choice

There are many types of surfactants that are used for synthesis of polymeric microparticles; such as sodium dodecyl sulfate (SDS), Tween80, Tween20, sodium cholate and PVA (Silva et al., 2013). PVA solutions are easily obtained by stirring PVA into water for few minutes at 90°C. The toxicity test showed that PVA has negative effects on animal skins and mucous membrane and the solution containing less than 5% PVA is not of any harm to fish (guppies) (Hallensleben, 2000).

6.1.6 Overview of Polyvinyl Alcohol (PVA)

Polyvinyl Alcohol (PVA) has been used as an emulsifier for preparation of micro and nano polymeric particles due to its excellent emulsifying and adhesive properties. PVA is also known for its high tensile strength; it is non-toxic, resistant to oil and grease and odorless. Besides, PVA has high oxygen and aroma barrier properties which depend on humidity. It has the melting point of 230°C for the fully hydrolyzed and 180–190°C for partially hydrolyzed classes (used in food production). It has been reported that PVA can undergo pyrolysis, during high temperatures and rapidly decompose above 200°C. The chemical structure of PVA is shown in Figure 33.

According to Nugent (2007), PVA formed a strong interface and demonstrated greater physical strength than the hydrogel because it is physically cross-linked. Such systems
have potential for a variety of localized controlled drug delivery applications, for example, as coatings for implantable devices. As for the PVA residual in PLGA nanoparticles, it was found even after three times washing, suggesting a strong surface adsorption of PVA on the surface of PLGA nanoparticles.

![Chemical structure of Polyvinyl alcohol (PVA)](image)

Figure 33. Chemical structure of Polyvinyl alcohol (PVA)

6.1.7 Adhesion of PVA on the surface of particles

Murakami (1999) proposed a model to express the adsorption of PVA molecules on the surface of PLGA particles, in which the hydroxyl groups of PVA molecules are fixed to the acetyl groups of PLGA via hydrophobic bonding (Figure 34). The excellent redispersibility of PLGA nanoparticles indicates that the surface of PLGA nanoparticles is stabilized by PVA molecules to prevent aggregation. The results showed that the PVA content and the particle size did not change even when the washing treatment was repeated six times. This is indicated that the surface of PLGA particles had strongly adsorbed a PVA layer.

![PVA interactions at the surface of PLGA nanoparticles](image)

Figure 34. PVA interactions at the surface of PLGA nanoparticles (with permission from Murakami et al., 1999)
6.1.8 Variables

The variables in the emulsion solvent evaporation technique that influence the final microsphere formation include: (i) nature and solubility of polymer in organic solvent; (ii) polymer concentration, composition and molecular weight; (iii) organic solvent used; (iv) concentration and nature of the stabilizer/surfactant; (v) temperature; (vi) stirring/agitation speed (shear rate) during emulsification process and; (vii) viscosity and volume ratio of the dispersed and continuous phase (Jain, 2000). Li et al (2008) was classified the variables in two main aspects: (1) the physico-chemical properties of materials, and (2) the preparation parameters that are involved in the process (Li et al., 2008). However, the focus of this study was on four variables: shear rate, mixing time, organic solvent and concentration of the surfactant. The goal of this study was to create a procedure for producing lignin microspheres from different sources with controlled sizes in spherical shape and narrow size distribution.

6.1.9 Theory of microspheres formation

6.1.9.1 Diffusion and evaporation of solvent

Mathematical models have been proposed for the solvent diffusion/evaporation from an open vessel system (Li et al., 2008; Li et al., 1995; Wang and Schwendeman, 1999). The model of diffusion/evaporation helps to understand the process of microspheres formation. As shown in Figure 35, two main steps are involved in the process of microspheres formation: solvent diffusion from drops of the dispersed phase to the continuous phase (F1) and solvent evaporation from continuous phase into the air (F2). Polymer microspheres begin to solidify by diffusion of the solvent from the drops in the dispersed phase to the continuous phase. The solidification step is completed by the total solvent evaporation from the continuous phase (Li et al., 2008).
The solvent diffusion/evaporation process contains three stages based on the concentration of the organic solvent in two phases. At the first stage, the dispersed phase is rich in solvent therefore the solvent is rapidly diffused into the continuous phase. As a result, the concentration of solvent inside the continuous phase ($C_s$) reaches the saturation level. This stage is very short (few seconds) and it can be neglected. In the second stage, the $C_s$ remains constant because the evaporated solvent is compensated with solvent diffused into the continuous phase. Initial quantities of the dispersed phase and of the continuous phase affect the duration of this stage. During the final stage, the polymer concentration in the continuous phase increases by decreasing the diffusivity of solvent from the dispersed phase to the continuous phase. The diffusion rate become smaller than evaporation rate, so $C_s$ begins to decrease (Li et al., 2008).

Based on Fick’s law and by assumption of zero solvent concentration above the surface of the continuous phase, the solvent evaporation would be (Li et al., 2008):

$$\frac{dM}{dt} = -A_{wa}.KC_s \quad (18)$$

where;

$M$ is the total mass of solvent in the reactor (kg)
$A_{wa}$ is the surface area of water-air interface (m$^2$)

t is time (s)

$K$ is evaporation constant (m/s)

$C_s$ is concentration of solvent in the continuous phase (kg/m$^3$).

During stage B, concentration of solvent in the continuous phase ($C_s$) is equal to the solubility of solvent in the continuous phase ($C_{sol}$).

$$\frac{dM}{dt} = -AKC_{sol} \quad (19)$$

Therefore, this stage of the solvent evaporation profile is linear.

6.1.9.2 Solidification of microsphere

Two mass transfers take place during the solidification of microsphere: solvent diffusion inside the drop and solvent diffusion at the boundary of the dispersed phase and the continuous phase (Figure 36). The mass flux in the centre of the drop is negligible. At the boundary, the mass transfer of solvent cause the decrease in the size of the drop. The size of drop is decreased during the solvent diffusion (Li et al., 2008).

![Figure 36. Schematic of mass transfers of solvent during solidification of microsphere](image)
6.1.10 Synthesis of hollow spheres

Hollow spheres have shown to have potential in a variety of applications ranging from controlled release to catalysis (Lasic, 1993; Yow and Routh, 2006). Hollow polymer spheres can be synthesized by using either chemical or physical methods (Kim and Yoon, 2004). Chemical methods are involved in chemical reactions through polymerization of monomers. For instance, McKelevy (2000) reported hollow cross-linked polystyrene spheres that can be templated from equilibrium vesicle phases (McKelvey et al., 2000). In another study from McKelvey (2002), divinyl benzene monomers were polymerized through free radical polymerization in the vesicle bilayer microstructure and results in a hollow polymeric sphere product (McKelvey and Kaler, 2002).

Physical methods do not involve chemical reactions during the formation of hollow polymer spheres. There are different ways to obtain hollow polymer spheres through physical methods: self-assembly of polymers in solutions, dispersing polymer solution droplets in immiscible media, and coating polymers on physically removable spherical templates (Kim and Yoon, 2004).

Hollow porous PLGA microspheres were prepared by double emulsion method when the organic phase to the volume ratio of the aqueous phase was 2:1 (Zhang et al., 2013b). Liu et al (2014) reported the fabrication, characterization and use of PLGA hollow microcapsules loaded with an anticancer drug for targeted drug delivery to cancer cells. PLGA hollow microcapsules were prepared by a double emulsion method; those having a size of 2.5 μm were stable (Liu et al., 2014).

6.1.11 Dynamic Light Scattering techniques

Photon correlation spectroscopy or dynamic light scattering is a valuable technique for particle size analysis of submicron particulates. This technique involves the focusing a monochromator laser beam on a particulate dispersion within a cell. Most of the laser beam passes straight through the sample, but some is scattered by the particles within the sample. A detector is used to measure the intensity of the scattered light. These fluctuations are the result of random Brownian motion of the particles, the rate of which
is inversely proportional to the particle size when the temperature is maintained at constant level. The technique provides information about the intensity distribution of the particles. It became routine particle-sizing tool in the study of several colloidal systems both micro and nanosize. The advantages associated with this method are the absence of traditional calibration, rapid sample analysis and minimal preparation of the sample (Zetasizer user manual, 2004).

6.1.11.1 Z-Average mean

The Z-Average mean or Z-Average size is the primary and most stable parameter produced by the dynamic light scattering technique. The Z-Average size is the best value to report in a quality control setting as it is defined in ISO 13321 as the “harmonic intensity averaged particle diameter”. The Z-average size is a hydrodynamic parameter and is only applicable to particles in a dispersion. It should be noted that the Z-average size is only comparable with the particle size measured by other techniques if the sample is spherical (or semi-spherical), monomodal and monodisperse (Malvern user manual, 2004).

6.1.11.2 Polydispersity Index (PDI)

Particles size distributions are one of the most important physical properties in a colloidal suspension. The polydispersity index is a dimensionless number calculated from a simple two parameter fit to the correlation data. This Index is scaled such that values smaller than 0.05 are highly monodisperse while values greater than 0.7 indicate very broad size distribution which is probably not suitable for the technique. Other size distribution between these two extremes work based on the parameters defined in the ISO 13321 standard (Malvern user manual, 2004).

6.1.12 Surface Charge (Zeta Potential)

Most liquids contain both positive and negative ions. When particles are suspended in liquid, liquid ions of opposite charge will be attracted to the surface of the particles. It is obvious that ions closer to the particles surfaces will be bound strongly to it, whereas ions that are positioned in outer layers of the particle will be loosely bound. A potential
exist between the particle surface and the dispersing liquid that vary accordingly with the distance from the ions to the particle surface. The potential is known as zeta potential.

Based on electrophoresis, the Zetasizer equipment measures the velocity by which the particles move towards the electrode of opposite charge. The technique is used in Laser Doppler Velocimetry (LDV). An incident beam is applied onto the electrophoretic cell and the resulting scattered light, produces a fluctuating intensity. The rate of fluctuation is proportional to the speed of the particles. A digital processor extracts the frequencies in the scattered light. In this equipment an optical modulator is present allowing an accurate reading, for millions of particles in a short period of time. Also due to the combination of both Laser Doppler Velocity and Phase analysis Light Scattering, the electro-osmotic effect, streaming potential and sedimentation potential are all minimized (Zetasizer manual, 2004).

The surface charge of the particles can be attributed to the dissociation of the polymeric groups and or to the absorption of ions or ionisable molecules from the dispersing phase (Mangenheim and Benita, 1991). The nature of the charge on the surface of particles can modify the biological response of these carriers, since it determines the type and nature of the interaction between the carriers and the active site (Douglas, 1987; Müller, 1991).

The stability of colloidal system, when dependent upon electrostatic stabilization, is related with the surface charge of the particles. Particles with high zeta potential value lead to a stable system, whereas a low zeta potential value results in particle aggregation (Harfield and Bunter, 1988).

Zeta potential is the difference in the electric potential existing between the dispersion medium and the first layer around the particle, referred to as the shear plane. The shear plane follows the movement of the particle. Zeta potential is dependent on the potential of the particles surface (Nerst potential). Zeta potential have another definition such as measure of the charge of the particles, the larger the absolute value of the zeta potential, the greater the amount of charge of charge at the surface. Therefore, the zeta
potential represents an index for particles stability. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of ±30 mV. This stability is important in preventing aggregation. This parameter can be used as indirect method for the determination of the surface charge of the particles. The relationship between the zeta potential of the particles and the stability of the colloid is presented in Table 25.

Table 25. Stability of colloids in relationship to the particle charge

<table>
<thead>
<tr>
<th>Zeta Potential (mV)</th>
<th>Stability of the colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to ±5</td>
<td>Rapid coagulation or flocculation</td>
</tr>
<tr>
<td>±10 to ±30</td>
<td>Incipient instability</td>
</tr>
<tr>
<td>±30 to ±40</td>
<td>Moderate stability</td>
</tr>
<tr>
<td>±40 to ±60</td>
<td>Good stability</td>
</tr>
<tr>
<td>More than ±60</td>
<td>Excellent stability</td>
</tr>
</tbody>
</table>

6.2 Experimental

6.2.1 Materials

Two isolated lignins from bioethanol biorefinery residues (L1) and hardwood kraft lignin (L2), and two commercial lignins (Indulin AT, softwood kraft pine lignin (L3) supplied by Westvaco Co., and Protobind 2000, non-wood (agricultural fibrous feedstock such as wheat straw) soda lignin (L4) supplied by ALM Private Limited) were used in this study.

Solvents were purchased from commercial sources: dichloromethane (DCM) (Caledon, Georgetown, ON, Canada), ethyl acetate (EA) (Caledon, Georgetown, ON, Canada), acetone (ACE) (Caledon, Georgetown, ON, Canada), tetrahydrofuran (THF) (Caledon, Georgetown, ON, Canada), pyridine (Caledon, Georgetown, ON, Canada), polyvinyl alcohol (PVA) (Sigma, St. Louis, MO), acetic anhydride (Caledon, Georgetown, ON,
Canada). All the chemicals used were analytical grade and used without further purification.

6.2.2 Acetylation of lignin

The method used in this study follows that used by Olarte (2011). 1.0 g lignin was mixed with 40 ml of pyridine-acetic anhydride (50-50%) solution. The mixture was allowed to react for 24 hours while mixing. The solids were re-precipitated with 150 ml of hydrochloric acid solution (pH = 1.0) and collected using a vacuum filtration technique. The solids were washed with some HCl solution and then with deionized water. The collected solids were dried at 40°C for overnight and stored in vials for further analysis.

6.2.3 Synthesis of lignin acetate microspheres at different conditions

Commercial non-wood soda lignin (Protobind 2000) was used in this study. To achieve a uniform lignin microspheres, the preparation parameters (i.e. shear rate, mixing time, and concentration of the surfactant) were designed based on the literature (Budhian et al., 2007; Freitas et al., 2005; Li et al., 2008; Sameni et al., 2009; Stewart et al., 2014). First, 10mg lignin acetate was dissolved in 1 mL organic solvent. The organic phase was transferred into 10 mL aqueous phase containing PVA with concentration of 0.0, 0.05, 0.1, 0.2, 0.5, 1 and 2 w/v%. The mixture was agitated with either magnetic stirrer (800 rpm and 1000 rpm) for 30 seconds or homogenizer (10,000-20,000 rpm) for 5, 10, 20 or 30 seconds. Then, the emulsion was transferred to a beaker containing 50 mL of distilled water and stirred for 2-3 h with magnetic stirrer at room temperature to allow the solvent to evaporate from the mixture. The particles collected by centrifugation for 10 min at 9000g, and washed twice with hot water to remove the surfactant. Samples were freeze-dried in and kept in a desiccator for further tests (Asrar and Ding, 2010; Silva et al., 2013).

Table 26 shows all the preparation parameters for synthesis of lignin acetate microspheres. The effect of each preparation parameter on the particle size was analyzed by using one-way ANOVA with significance when p<0.05.
6.2.4 Preparation of lignin microspheres and lignin acetate microspheres using different lignins

After optimization of the parameters (i.e. shear rate, mixing time, and concentration of the surfactant), lignin microspheres were synthesized with isolated lignins (or lignin acetates) from different sources. The organic phase was prepared by dissolving 10 mg of soluble part of lignin (or lignin acetate) in 1 mL organic solvent. The soluble part of lignin in organic solvent was collected by centrifuging and removing the insoluble part.
Then, the organic phase was intermixed with 10 mL PVA (0.2%) by using homogenizer at 10,000rpm for 30 seconds and the transferred into 50 mL water. Samples were collected in a similar way as explained in the section 6.2.3. Figure 37 illustrates the overall process for the synthesis of the lignin microspheres.

6.2.5 Preparation of lignin acetate hollow spheres

At low shear rate (1000 rpm) a portion of particles (hollow spheres) remained on the surface of the water after centrifugation due to their lower density than water. Particles were collected from the surface of water, washed with distilled water and dried in the freeze drier.
Figure 37. Method for synthesis of lignin microspheres: (1) lignin-containing organic solvent is mixed with an aqueous PVA solution to make an oil-in-water emulsion, (2) Diffusion of organic solvent from organic phase to aqueous phase, (3) washing and collection the particles, and finally (4) drying samples by using freeze drier.
6.2.6 Determination of size and size distribution of lignin particles by using Dynamic light scattering (DLS) technique

The average diameter of microspheres and polydispersity index (PDI) were determined by using ZETASIZER NANO ZS Malvern Instrument (Malvern, United Kingdom), at 25.0±0.1 °C. The colloidal dispersions of lignin microspheres were prepared at 0.1% concentration of aqueous solution. The size distribution graphs were obtained based on the relative intensity of scattered light on the hydrodynamic diameter of lignin microspheres. The relative intensity peaks were normalized (the intensity of highest peak normalized to unity) for all samples. At least three measurements were carried out for each test.

6.2.7 Determination of zeta potential by using DLS technique

The zeta-potentials of lignin microspheres were determined using ZETASIZER NANO ZS Malvern Instrument (Malvern, United Kingdom), at 25.0±0.1 °C. A colloidal dispersion of lignin microspheres was prepared in an aqueous solution with concentration of 0.01%. At least three measurements were carried out for each test.

6.2.8 Morphology of lignin particles by Scanning Electron Microscopy

The morphology of microspheres was observed under scanning electron microscopy (SEM). SEM was used to characterize the morphology of the lignin particles. Samples were sputter-coated by a fine gold layer (10 nm) and observed on a JEOL field emission microscope (5 kV). Diameters of the lignin particles were measured by using ImageJ software. The mean diameter of each sample was estimated based on the measurements of 100 randomly selected particles.

6.2.9 The yield percentage determination

The yield percentage of the obtained lignin microspheres or lignin acetate microspheres was determined by measuring the dry weight of the particles after the filtration of the suspension using a Buchner funnel with 11 µm pore filter paper.
6.2.10 Mixture Stability test

The stability of the lignin microspheres mixture was studied by analyzing the particles over time. Particle size, PDI and zeta-potential of ACL4DCM and ACL4EA particles were determined on the first day of preparation and after 15, 25, 35 and 60 days in a neutral suspension at room temperature. The stability test for other formulations was determined only on the first day and after 60 days in a neutral suspension at room temperature. The results were reported as mean ±standard deviation (±SD). Statistical analysis were performed by using one-way analysis of variance (ANOVA) with significance (p<0.05), highly significant (p<0.01) and very significant (p<0.001). The morphology of the particles on the 60th day was studied by analyzing SEM images.

6.3 Results and discussion

6.3.1 The effect of preparation parameters on the lignin particles formation

6.3.1.1 The influence of mixing shear rate

Generally the size of droplets in an emulsion is inversely related to the magnitude of shear stresses. Therefore, smaller microspheres are formed by increasing the shear rate. It is reported that increasing stirring speed produced smaller particles using emulsion solvent evaporation technique (O'Donnell and McGinity, 1997). As the speed of the motor or the power of the sonicator is increased, the size of the dispersed droplets decreases (Ansari et al., 2012). Therefore, if high shear is produced by homogenizer or sonicator, the droplets become much smaller than the droplets in the emulsion produced by conventional agitation. This phenomenon strongly supports the concept that the stronger shear forces and increased turbulences that are generated at high stirring speed could breakdown the droplets into smaller sizes (Freitas et al., 2005).

Figure 38a shows the particle size distribution of lignin acetate microspheres that were prepared at low shear (800 rpm and 1000 rpm) and high shear rates (10,000 rpm). The low shear was applied by a magnetic stirrer and high shear by a homogenizer. The size of the particles prepared using a magnetic stirrer was determined by imageJ software (Table 27). Particles were formed in larger size and in wider distribution by using
magnetic stirrer. The average particle size at 800 rpm and 1000 rpm was 13.6 µm and 10.6 µm, respectively.

Figure 38b shows the size distribution of lignin acetate microspheres prepared by homogenizer at four different shear rates between 10,000 rpm and 20,000 rpm. All samples showed unimodal size distribution. However, it seems that the distributions of lignin acetate microspheres were slightly shifted to lower sizes at higher shear rate.

![Figure 38a](image1.png)  ![Figure 38b](image2.png)

Figure 38. Particle size distribution of lignin acetate microspheres at different shear rate; Particle size was determined by a) imageJ software and b) DLS technique

Table 27. Minimum, maximum and mean particle size prepared by using magnetic stirrer and measure by imageJ software

<table>
<thead>
<tr>
<th>Shear rate (rpm)</th>
<th>Particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
</tr>
<tr>
<td>800</td>
<td>3.8</td>
</tr>
<tr>
<td>1,000</td>
<td>2.0</td>
</tr>
<tr>
<td>10,000</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Figure 39 shows the effect of shear rate on the average particle size and PDI of the lignin acetate microspheres. It is clear that the average size of microspheres was decreased from 1075 nm to 886 nm when the shear rate was increased from 10,000 rpm to 20,000 rpm. Significance of the influence was statistically confirmed by one-way ANOVA ($p < 0.05$). Although, the size distribution became slightly narrower by increasing the shear rate from 10,000 rpm to 15,000 rpm, the value of PDI was increased at very high shear rates (20,000 rpm). It has been reported that the particle size distribution of PLGA and Eudragit RS microspheres decreased when stirring speed increased (Gabor, 1999; Mateovic et al., 2002). SEM images (Figure 40) shows the obvious differences between the effects of the different shear rates on the particle size.

![Graph](image)

Figure 39. Average particle size (Z-Ave) and polydispersity (PDI) of lignin acetate microspheres at different shear rate applied by homogenizer.
Figure 40. SEM images of lignin acetate microspheres which prepared by using magnetic stirrer at low shear (800 rpm and 1000 rpm) and homogenizer at high shear rate (10,000-20,000 rpm).
Many other factors related to agitation also have an influence on the size of the microspheres, such as the geometry of the reactor, the number of impellers and the impeller’s diameter (Maa and Hsu, 1996). Based on the Kolmogoroff/Hinze model (Hinze, 1955) the correlation between the agitation rate and the diameter of the agitator could be expressed as:

\[
\frac{d_{\text{max}}}{D} = C_1 \left( \frac{\rho_c N_t^2 D^3}{\sigma} \right)^{-3/5} \tag{20}
\]

Where;

\(d_{\text{max}}\) is the largest drop size which can be formed under turbulence (m),

\(D\) is the diameter of the agitator (m)

\(\rho_c\) is the density of continuous phase (kg/m\(^3\)),

\(N_t\) is the agitation rate (turns/s)

\(\sigma\) is the interfacial tension between the continuous phase and the dispersed phase (N/m)

\(c_1\) is a constant value which depends on the factors linked to the agitation conditions

If we assume a constant density for the continuous phase and a constant interfacial tension between the continuous phase and the dispersed phase, then;

\[
\frac{d_{\text{max}}}{D} = C_2 (N^2 D^3)^{-3/5} \tag{21}
\]

where \(C_2\) is a constant.

From (20 and 21, it is clear that the maximum size of microspheres is decreased by increasing the agitation rate (Gabor, 1999; Mateovic et al., 2002; Yang et al., 2001).
Figure 41 shows the relationship between the largest particle size of lignin acetate microspheres with the diameter of the agitator and the agitation rate.

![Graph showing the relationship between the largest particle size of lignin acetate microspheres with the diameter of the agitator and the agitation rate.](image)

Figure 41. The relationship between the diameter of the agitator (D) and the agitation rate (N) with the maximum size of the lignin acetate microspheres ($d_{\text{max}}$).

6.3.1.2 Formation of lignin acetate hollow spheres

Figure 42 shows SEM images of the lignin acetate hollow spheres. The average diameter and the thickness of the lignin acetate hollow spheres were determined by imageJ software on the SEM images. It was found that the thickness of the hollow spheres was about 1-3 µm, and the average particle size was about 58 µm (Figure 43).

A hollow structure of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) was also observed by using Tween80 and PVA as emulsifiers (Khang et al., 2001). PLGA hollow microcapsules loaded with an anticancer drug for targeted drug delivery to cancer cells was reported by Liu et al (2014). These PLGA hollow microcapsules were synthesized by a double emulsion technique having a size of 2.5 µm (Liu et al., 2014). Hollow porous PLGA microspheres were also prepared by double emulsion technique at organic phase to aqueous phase ratio of 2:1 (Zhang et al., 2013b).
Figure 42. SEM images of lignin acetate hollow spheres

Figure 43. Particle size distribution of lignin acetate hollow spheres
Formation of lignin hollow spheres was directly related to the shear rate of the mixer. At lower energy dispersion lignin particles were formed in bilayer to reduce the Gibbs free energy (Figure 44).

![High shear rate diagram](image)

**High shear rate:**

![Low shear rate diagram](image)

**Low shear rate:**

Figure 44. Formation of lignin microspheres and hollow spheres at different shear rate

6.3.1.3 The role of surfactant in microspheres formation

Surfactants play an important role in the formulation of microspheres and in their resulting shape and size. The main role of the surfactant is to prevent the emulsion droplets from coalescing. The surfactant molecules are located in the interface between the aqueous phase and organic phase. The concentration and properties of the surfactants will affect the total surface area of the particles and may change the final particle size (Manchanda et al., 2010). Figure 45 illustrates the surfactant stabilized lignin microspheres and hollow spheres. The hollow sphere structure consists of an aqueous core, lignin acetate layer and two PVA monolayers.
6.3.1.4 The influence of surfactant concentration

Figure 46 shows the particle size distribution of lignin acetate microspheres that were prepared at different PVA concentrations (0.05 - 2.0% w/v). Unimodal distribution was observed for all cases. The size distribution was shifted to smaller particles by increasing the PVA concentration from 0.05% to 1%. However, by increasing the PVA to 2%, the size distribution was moved to larger particles.
Figure 46. Particle size distributions of lignin acetate microspheres at different PVA concentration

Figure 47 shows that the average particle size of lignin acetate microspheres was decreased by addition of PVA from 0.05% to 1%, and then increased by addition of 2% PVA. Therefore, the smallest particle size (744 nm) was obtained when 1% PVA was used for particles formation. However, the polydispersity of particles at 1% PVA was the highest (PDI=0.22) in comparison with other samples. According to ANOVA, the PVA concentration is found to have a significant influence on the lignin acetate particle size (p<0.05).

Feritas and co-workers (Freitas et al., 2005) found that the polymeric particle size is reduced with an increasing the surfactant concentration. Silva et al (2013) reported that the PVA concentrations below 1% led to a larger particle size of poly(lactic-co-glycolic acid) microspheres (Silva et al., 2013). Smaller particles have a higher total interfacial area compared to the large particles, thus they require a higher concentration of the surfactant. Therefore, the addition of higher surfactant concentration to the solution results in decreased particles size. An increase (Zweers et al., 2004) and decrease (Allemann et al., 1992) in size of poly(lactic-co-glycolic acid) nanoparticles at high PVA concentration have been reported. These contradictory findings were clarified by
Budhian (Budhian et al., 2007) who proposed two competing effects at high PVA concentration. The size of the particles decreases due to enhanced interfacial stabilization while the size of the particles increases due to increased viscosity of the aqueous phase.

Figure 47. Average particle size and PDI of lignin acetate microspheres at different PVA concentration

SEM images show the effect of surfactant concentration on the morphology of lignin acetate microspheres (Figure 48). A comparison between SEM images showing the formation of lignin acetate particles with surfactant was completely in spherical shape and had a smooth surface. Absence of surfactant causes particle shrinkage and a rough surface on the particles.
Figure 48. SEM images of lignin acetate microspheres at different PVA concentration (0.0-2.0%). Agitation rate was 10,000 rpm for all cases, unless stated on the image.
6.3.1.5 The influence of mixing time

Figure 49 presents size distribution of lignin acetate microspheres that were prepared at different mixing times (5, 10, 20 and 30 seconds) by using homogenizer at 10,000rpm and constant PVA concentration (0.2%w/v). It shows uniform size distribution for all samples, but obvious differences of particle sizes was observed between samples. Larger particles were formed during shorter mixing time. The portion of large particles was much higher when shorter time was applied in the process.

![Figure 49. Particle size distribution of lignin acetate microspheres at different time for agitation](image)

Figure 50 shows the average particle size and PDI of lignin acetate microspheres at different mixing time. The average particle size was 1767 nm, 1291 nm, 1062 nm and 1075 nm at 5, 10, 20 and 30 seconds of mixing time, respectively. ANOVA showed significant difference between lignin acetate particle size at different mixing times (p<0.05). PDI was decreased from 0.216 to 0.11 when the mixing time increased from 5 to 30 seconds.

Short mixing time yields coarse particles due to less magnitude of shear stress applied, while at longer time, the energy density increases directly by increasing the shear stresses and results in more efficient droplet breakdown (Budhian et al., 2007).
Therefore, increasing the mixing time decreases the particles mean size due to reduction of emulsion droplets through sufficient shear forces.

Figure 51 shows SEM images of lignin acetate microspheres at different mixing time.

![Graph showing average particle size and polydispersity index (PDI) of lignin acetate microspheres at different mixing time]

Figure 50. Average particle size and polydispersity index (PDI) of lignin acetate microspheres at different mixing time
Figure 51. SEM images of lignin acetate microspheres at different mixing time (the scale bar is 5 μm)

6.3.1.6 The effect of organic solvent on the particle formation

SEM images show the formation of lignin acetate microsphere when DCM, ACE, EA and THF were chosen as organic solvents. Homogenizer was used as agitator with 10,000 rpm, mixing time was fixed at 30 seconds, and PVA concentration was 0.2 w/v%. Although lignin acetate was completely soluble in all four organic solvents, lignin acetate microspheres were formed only in DCM and EA.
Figure 52. SEM images of lignin acetate microspheres at different organic solvents (the scale bar is 5 μm)

Figure 53 illustrates the size distribution of the lignin acetate microspheres. A comparison between the size distributions indicates a wider size distribution with EA compared with DCM.
Table 28 shows the average size, PDI, and zeta-potential of the lignin acetate microspheres when the organic solvent was EA and DCM. The average size of the particles was 1881 nm and 1075 nm for EA and DCM, respectively. PDI was in narrower distribution for DCM (0.118) in comparison to EA (0.173). The results show that the zeta-potential of the lignin acetate microspheres with EA is -45.5 mV while with DCM is -36.7 mV. The value of zeta-potential depends on the chemicals involved in the synthesis process (Patil et al., 2007). The negative zeta-potential is caused by the residue of the PVA surfactant on the particles surface (Chumakova et al., 2008), residue of the solvent and surface free carboxylic acid groups on the lignin acetate.
Table 28. Average size, PDI and zeta-potential of lignin acetate microspheres in EA and DCM

<table>
<thead>
<tr>
<th>Organic solvent</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
<th>zeta-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>1881 (±46.5)</td>
<td>0.173 (±0.030)</td>
<td>-45.5 (±2.13)</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1075 (±6.9)</td>
<td>0.118 (±0.018)</td>
<td>-36.7 (±0.46)</td>
</tr>
</tbody>
</table>

It is clear that the formation of particles mainly depended on the physical properties of the organic solvent. Physical properties of the selected organic solvents are presented in Table 29. ACE and THF diffuse rapidly from the dispersed phase into the aqueous phase due to their miscibility in water. Therefore, lignin acetate was dispersed in aqueous phase in irregular shapes before it was formed and stabilized by the surfactant molecules. On the other hand, DCM is immiscible in water and the solubility of EA is low in water (8.3 g/100mL). Therefore, DCM and EA remained in the emulsion droplets for a while before diffusing into the aqueous phase. The results indicate that lignin acetate particles are formed and solidified in uniform size and shape by using DCM and EA in the process.

Interfacial tension of DCM is higher than EA, and it is immiscible in water. Therefore, DCM resulted in successful formation of smaller lignin acetate microspheres with narrow size distribution in comparison with EA.

The higher solubility of EA in water may result in significant aggregation leading to larger particles. In addition, higher density of DCM than water may delay the solvent removal from the droplets and increase the uniformity of the particles. Lower viscosity of DCM than EA may also affect the particle size.

Dichloromethane (chlorinated solvents that challenge human safety and environmental concern) have been widely used as a good organic solvent in emulsion solvent evaporation technique. In order to reduce the use of these toxic solvents, many attempts have been made to prepare the polymer microspheres using a solvent with
lower toxicity, such as ethyl acetate, as the dispersing solvent. The effect of ethyl acetate as a dispersing solvent was also studied in the production of different polymers such as PLGA microspheres (Soppimath and Aminabhavi, 2002).

Table 29. Some physical properties of selected organic solvent (Patil et al., 2007; Sah, 1997)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Bp (°C)</th>
<th>Density (g/cm³) at 20 °C</th>
<th>Solubility in water (wt%) at 20-25°C</th>
<th>Viscosity (Cp)</th>
<th>Interfacial tension (dyne/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>39.8</td>
<td>1.3255</td>
<td>1.32</td>
<td>0.44</td>
<td>28.3</td>
</tr>
<tr>
<td>EA</td>
<td>76.7</td>
<td>0.9018</td>
<td>8.7</td>
<td>0.46</td>
<td>1.3</td>
</tr>
<tr>
<td>ACE</td>
<td>56.0</td>
<td>0.7910</td>
<td>miscible</td>
<td>0.32</td>
<td>-</td>
</tr>
<tr>
<td>THF</td>
<td>66.0</td>
<td>0.8892</td>
<td>miscible</td>
<td>0.48</td>
<td>-</td>
</tr>
</tbody>
</table>

6.3.1.7 Stability of the lignin acetate microspheres suspension

Table 30 shows the particle size, PDI and zeta-potential of the microspheres on the first day of preparation and after 15, 25, 35 and 60 days in a neutral suspension at room temperature. According to ANOVA analysis, lignin acetate microspheres prepared with DCM were found to be stable up to 35 days (p>0.05), while the particle size was slightly increased at 60 days (p<0.05). ANOVA results showed that the lignin acetate microspheres prepared with EA were slightly enlarged in the first 10 days of the stability test (p<0.05) and continued to 60 days of the test.

Zeta-potential drastically decreased over time when compared to those of freshly prepared samples. Zeta-potential is a measure of the particle stability with greater negative or positive charge causing more repulsion between the particles and reducing
the particles aggregation (Chumakova et al., 2008). The reduction of Zeta-potential over time can be attributed to the slow diffusion of the organic solvent to the aqueous phase.

The zeta-potential of PVA particles alone (1% w/v) was reported to be -8 mV at neutral pH (Ravi Kumar et al., 2004), therefore adhesion of PVA on the particles may alter the particles zeta-potential. Adsorption of PVA on the surface of lignin microspheres may be expressed by the Murakami (1999) model (Murakami et al., 1999); that is, the hydroxyl groups of PVA molecules are fixed to the acetyl groups of Poly(lactic-co-glycolic acid) via hydrophobic bonding (See Section 6.1.7).
Table 30. Average size, PDI and zeta-potential of lignin acetate microspheres prepared in dichloromethane and ethyl acetate subjected to stability test at room temperature over time. Mean value (±Standard Deviation)

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
<th>zeta -potential (mV)</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
<th>zeta -potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1075 (±6.9)</td>
<td>0.118 (±0.018)</td>
<td>-36.7 (±0.46)</td>
<td>1881 (±46.5)</td>
<td>0.173 (±0.030)</td>
<td>-45.5 (±2.13)</td>
</tr>
<tr>
<td>15</td>
<td>1078 (±10.0)</td>
<td>0.118 (±0.012)</td>
<td>-30.3 (±0.22)</td>
<td>1957 (±10.0)</td>
<td>0.178 (±0.027)</td>
<td>-40.1 (±3.11)</td>
</tr>
<tr>
<td>25</td>
<td>1085 (±5.3)</td>
<td>0.152 (±0.020)</td>
<td>-26.4 (±0.37)</td>
<td>2004 (±14.5)</td>
<td>0.190 (±0.033)</td>
<td>-39.3 (±1.35)</td>
</tr>
<tr>
<td>35</td>
<td>1094 (±16.3)</td>
<td>0.173 (±0.016)</td>
<td>-20.7 (±0.92)</td>
<td>2117 (±50.5)</td>
<td>0.197 (±0.025)</td>
<td>-37.8 (±0.95)</td>
</tr>
<tr>
<td>60</td>
<td>1137 (±21.5)</td>
<td>0.152 (±0.032)</td>
<td>-22.8 (±0.91)</td>
<td>2184 (±22.1)</td>
<td>0.142 (±0.060)</td>
<td>-39.4 (±1.90)</td>
</tr>
</tbody>
</table>
SEM images of the lignin acetate microspheres in DCM and EA show that the particles remained in stable shape without shrinking or collapsing after 60 days (Figure 54).

Figure 54. Lignin acetate microspheres after 60 days in neutral suspension (the scale bar is 10 μm)

6.3.2 Synthesis and characterization of lignin acetate microspheres from different sources

Lignin acetate microspheres were prepared from different isolated lignins based on the optimum preparation parameters which were described in the previous section 6.3.1. According to this method, the homogenizer was used as agitator with 10,000 rpm, mixing time was fixed at 30 seconds, PVA concentration was prepared at 0.2 w/v% and organic solvent were either DCM or EA.

6.3.2.1 Synthesis of lignin acetate microspheres in DCM

The reproducibility of the process was examined by producing ACL4-DCM three times under controlled conditions (Figure 55). Table 31 shows the average size and the PDI of the lignin microspheres which synthesized by using the homogenizer at 10,000 rpm for 30 seconds and 0.2 %w/v PVA concentration. ANOVA showed significant difference between all samples (p<0.05), however, there is no significant difference between sample #2 and #3 (p>0.05).
Figure 55. Comparison between lignin acetate microspheres prepared with the same conditions

Table 31. Reproducibility of lignin microspheres by using controlled parameters through emulsion solvent evaporation technique

<table>
<thead>
<tr>
<th>Test</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL4-DCM #1</td>
<td>1331 (±21.0)</td>
<td>0.121 (±0.034)</td>
</tr>
<tr>
<td>ACL4-DCM #2</td>
<td>1075 (±6.9)</td>
<td>0.118 (±0.018)</td>
</tr>
<tr>
<td>ACL4-DCM #3</td>
<td>1281 (±20.0)</td>
<td>0.110 (±0.065)</td>
</tr>
</tbody>
</table>

Figure 56 shows the SEM images of lignin acetates microspheres when DCM was chosen as organic solvent. It is clear that all four samples were formed in spherical shape and with relatively uniform size distribution.
Figure 56. SEM images of lignin acetate microspheres when DCM was chosen as organic solvent in the method (the scale bar is 5 μm).

Size and size distribution of the particles were determined by the DLS technique (Figure 57). All four samples were formed in uniform and unimodal distribution in the range between 600 nm to 4000 nm.
Figure 57. Particle size distributions of lignin acetate microspheres isolated from different sources

Table 32 shows the average size and PDI of the lignin acetate microspheres. The average size of the particles was in a close range about 1280nm to 1376nm and PDI was in narrow distribution from 0.100 to 0.170. Although lignin samples are different in their molecular weights, it seems that the formation of lignin acetate microspheres was independent of the molecular weight of the lignin.

Table 32. Average particle size and polydispersity index of lignin acetate microspheres prepared in DCM

<table>
<thead>
<tr>
<th>Sample</th>
<th>Z-Ave(d.nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL1-DCM</td>
<td>1280 (±29.0)</td>
<td>0.100 (±0.061)</td>
</tr>
<tr>
<td>ACL2-DCM</td>
<td>1376 (±31.0)</td>
<td>0.121 (±0.026)</td>
</tr>
<tr>
<td>ACL3-DCM</td>
<td>1296 (±7.6)</td>
<td>0.170 (±0.028)</td>
</tr>
<tr>
<td>ACL4-DCM</td>
<td>1331 (±21.0)</td>
<td>0.121 (±0.034)</td>
</tr>
</tbody>
</table>
The charge of particles was determined by DLS technique (Figure 58). The results showed that the zeta potential of all four lignins samples was between -20 mV to -30 mV when lignin microspheres were prepared in DCM. According to ANOVA, there was no significant difference (p>0.05) between the zeta potential of different lignin microspheres (ACL1-DCM, ACL2-DCM, ACL3-DCM, ACL4-DCM).

![Zeta potential of lignin acetates microspheres](image)

Figure 58. Zeta potential of lignin acetates microspheres

6.3.2.2 Synthesis of lignin acetate microspheres in EA

Figure 59 shows the SEM images of lignin acetate microspheres when ethyl acetate was used as dispersing solvent in the emulsion solvent evaporation technique. Although particles were formed in uniform spherical shape, it seems that the average size and size distribution is different for each lignin.
Figure 59. SEM micrographs of lignin acetate microspheres when EA was chosen as organic solvent in the method (the scale bar is 5 μm)

Figure 60 shows the size distribution of lignin acetate microspheres when EA was used as dispersing solvent. Although the particle size distribution was uniform for all types of lignin, ACL1 and ACL4 were formed with a higher size distribution than ACL2 and ACL3.
Figure 60. Size distribution of lignin acetate when EA was used as dispersing solvent

Table 33 shows the average particle size and the PDI of the lignin acetate microspheres in EA. The largest particle size was formed for ACL1 which was about 3433 nm and the smallest particle size was 1196 nm for ACL3. PDI was in a range from 0.130 to 0.233.

Table 33. Average size and PDI of lignin acetate microspheres, EA was used as organic solvent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL1-EA</td>
<td>2545 (±70.6)</td>
<td>0.082 (±0.063)</td>
</tr>
<tr>
<td>ACL2-EA</td>
<td>1323 (±23.0)</td>
<td>0.163 (±0.014)</td>
</tr>
<tr>
<td>ACL3-EA</td>
<td>1196 (±16.4)</td>
<td>0.233 (±0.023)</td>
</tr>
<tr>
<td>ACL4-EA</td>
<td>1881 (±46.5)</td>
<td>0.173 (±0.030)</td>
</tr>
</tbody>
</table>
Zeta-potential of lignin acetate microspheres from different sources is illustrated in Figure 61. The particle charge for all samples showed a high negative charge which was in the range from -43 mV to -48 mV. Results of one-way ANOVA reveal no significant difference (p>0.05) between the zeta potential of lignin microspheres.

Lignin acetate microspheres were formed in both DCM and EA organic solvents. However, the particles formed in DCM were more uniform and smaller in size than in EA. The reason for this phenomenon might be explained by the differences in the physical properties of two organic solvents. DCM is immiscible in water and the solubility of EA is low in water (8.3 g/100mL). Therefore, the DCM remained in the emulsion droplets for longer time than EA, before diffusion into the aqueous phase. The results showed that lignin acetate particles that were prepared with DCM were more uniform in size and shape than EA.
Preparation and characterization of lignin microspheres in EA

Although DCM was found to be a good solvent for synthesis of lignin acetate microspheres, it was not a suitable solvent for synthesis of lignin microspheres due to very low solubility of lignin in DCM. Table 34 shows the solubility of lignins in ethyl acetate which was determined in previous chapter (See Figure 23). Solubility of all lignin samples in EA was higher than in DCM. It is clear that the solubility of L2 in EA is highest compared with other lignins.

<table>
<thead>
<tr>
<th>Lignin</th>
<th>Solubility in EA</th>
<th>Solubility in DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>23.7 (±3.2)</td>
<td>10.0 (±5.0)</td>
</tr>
<tr>
<td>L2</td>
<td>60.6 (±1.8)</td>
<td>20.5 (±0.5)</td>
</tr>
<tr>
<td>L3</td>
<td>8.8 (±0.9)</td>
<td>0.7 (±0.1)</td>
</tr>
<tr>
<td>L4</td>
<td>42.7 (±4.7)</td>
<td>4.1 (±1.1)</td>
</tr>
</tbody>
</table>

SEM images of lignin microspheres are shown in Figure 62. Microspheres were formed in EA when the solubility of the lignin was high. Since L1 and L3 showed very low solubility in EA, the lignin microparticles were not well-formed.
Figure 62. SEM micrographs of lignin microspheres. EA was chosen as organic solvent in the method (the scale bar is 5 μm).

Table 35 shows the average size and PDI of lignin microspheres that were prepared in EA. Only L2-EA was formed with low PDI.

Table 35. Average size and PDI of lignin acetate microspheres, EA was used as organic solvent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Z-Ave (d.nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-EA</td>
<td>1279 (±41.8)</td>
<td>0.425 (±0.025)</td>
</tr>
<tr>
<td>L2-EA</td>
<td>1152 (±28.5)</td>
<td>0.233 (±0.012)</td>
</tr>
<tr>
<td>L3-EA</td>
<td>3378 (±250)</td>
<td>1.0 (Rejected)</td>
</tr>
<tr>
<td>L4-EA</td>
<td>875 (±15.1)</td>
<td>0.431 (±0.040)</td>
</tr>
</tbody>
</table>
The size distributions of the lignin microparticles are illustrated in Figure 63. A uniform size distribution for L2 lignin is seen, while L1 and L4 formed in bimodal distribution. The particles in L3 sample were not detected by the DLS due to very high dispersity of the sample.

![Size distribution of lignin microspheres](image)

Figure 63. Size distribution of lignin microspheres when EA was used as dispersing solvent

The zeta-potentials of lignin microspheres are illustrated in Figure 64. It shows that the lignin microspheres have lower charge than lignin acetate microspheres. The obvious differences between the surface charge of the lignin and lignin acetate microspheres mostly is due to the functional groups of the lignins. During the acetylation process, hydroxyl groups are converted to acetyl groups. However, the remaining part of the surfactant on the particle surface may also affect the particle charge. ANOVA results showed significant difference (p<0.05) between the zeta potential of lignin microspheres.
Figure 64. Zeta potential of lignin microspheres (L3 was rejected by the DLS analysis)

6.3.2.4 Yield percentages of microspheres

The yield percentages of the particles obtained from different lignins are shown in Table 36. All lignin acetate microspheres were obtained in high percentages, while lignin microspheres were obtained at lower percentage. Only L2 and L4 with 88.8% and 55.8% were obtained in this process. The yield percentages for L1 and L3 were very low. Although acetylated L2 shows potential for synthesis of lignin acetate microspheres with both organic solvents, unmodified L2 lignin also showed promising results for synthesis of lignin microspheres.
Table 36. The yield percentage of the particles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL1</td>
<td>DCM</td>
<td>98.2</td>
</tr>
<tr>
<td>ACL2</td>
<td>DCM</td>
<td>97.5</td>
</tr>
<tr>
<td>ACL3</td>
<td>DCM</td>
<td>98.1</td>
</tr>
<tr>
<td>ACL4</td>
<td>DCM</td>
<td>96.4</td>
</tr>
<tr>
<td>ACL1</td>
<td>EA</td>
<td>90.3</td>
</tr>
<tr>
<td>ACL2</td>
<td>EA</td>
<td>95.6</td>
</tr>
<tr>
<td>ACL3</td>
<td>EA</td>
<td>96.3</td>
</tr>
<tr>
<td>ACL4</td>
<td>EA</td>
<td>97.6</td>
</tr>
<tr>
<td>L1</td>
<td>EA</td>
<td>1.3</td>
</tr>
<tr>
<td>L2</td>
<td>EA</td>
<td>88.8</td>
</tr>
<tr>
<td>L3</td>
<td>EA</td>
<td>0</td>
</tr>
<tr>
<td>L4</td>
<td>EA</td>
<td>55.8</td>
</tr>
</tbody>
</table>

6.3.2.5 Stability of the lignin microspheres suspension

Table 37 illustrates the stability of the lignin microspheres mixture after 60 days in aqueous suspension. A very significant increase in particle size was observed for almost all samples except the ACL2EA (P>0.01), ACL2DCM (p>0.001) and L2EA (p>0.001). It shows that L2 had the most stable microspheres in both untreated and acetylated forms. The mixture of unmodified L2 microspheres showed greater stability than other unmodified lignin microspheres. SEM images of the lignin acetate microspheres showed that the particles remained in spherical shape after 60 days in aqueous solution. However some agglomeration of the small particles was observed in some samples (Figure 65).
Table 37. The average size of microspheres in the first day and after 60 days in aqueous suspension. P-values is for Z-Ave of the lignin microspheres.

<table>
<thead>
<tr>
<th>Sample</th>
<th>First day</th>
<th>60 days</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-Ave</td>
<td>PDI</td>
<td>Z-Ave</td>
</tr>
<tr>
<td>ACL1-DCM</td>
<td>1280 (±29.0)</td>
<td>0.100 (±0.061)</td>
<td>1621.3(±21.1)</td>
</tr>
<tr>
<td>ACL2-DCM</td>
<td>1376 (±31.0)</td>
<td>0.121(±0.026)</td>
<td>1509.0(±35.5)</td>
</tr>
<tr>
<td>ACL3-DCM</td>
<td>1297 (±7.6)</td>
<td>0.170(±0.028)</td>
<td>1701.3(±18.1)</td>
</tr>
<tr>
<td>ACL4-DCM</td>
<td>1331(±21.0)</td>
<td>0.121(±0.034)</td>
<td>1617.3(±34.8)</td>
</tr>
<tr>
<td>ACL1-EA</td>
<td>2545 (±70.6)</td>
<td>0.082(±0.063)</td>
<td>Rejected</td>
</tr>
<tr>
<td>ACL2-EA</td>
<td>1323 (±23.0)</td>
<td>0.163(±0.014)</td>
<td>1389.0(±12.3)</td>
</tr>
<tr>
<td>ACL3-EA</td>
<td>1196 (±16.4)</td>
<td>0.233(±0.023)</td>
<td>1422.7(±16.6)</td>
</tr>
<tr>
<td>ACL4-EA</td>
<td>1881(±46.5)</td>
<td>0.173(±0.030)</td>
<td>2184.3(±22.1)</td>
</tr>
<tr>
<td>L1-EA</td>
<td>1279 (±41.8)</td>
<td>0.425(±0.025)</td>
<td>Rejected</td>
</tr>
<tr>
<td>L2-EA</td>
<td>1152(±28.6)</td>
<td>0.233(±0.012)</td>
<td>1452.3(±92.1)</td>
</tr>
<tr>
<td>L3-EA</td>
<td>Rejected</td>
<td>-</td>
<td>Rejected</td>
</tr>
<tr>
<td>L4-EA</td>
<td>875(±15.1)</td>
<td>0.431(±0.040)</td>
<td>Rejected</td>
</tr>
</tbody>
</table>
Figure 65. SEM images of lignin acetate microspheres after 60 days in 0.1% aqueous suspension (arrow shows the agglomerations) (the scale bar is 5 μm)
Figure 66 shows the SEM images of the lignin microspheres after 60 days in 0.1% aqueous suspension. It is clear that the L2EA particles remained uniform, while some agglomerations were observed in L4EA sample on the 60th day of the stability test. SEM images of L1EA and L3EA samples showed very low percentage and high agglomeration of microspheres which was the same as the first day of the preparation.

Figure 66. SEM images of lignin microspheres after 60 days in 0.1% aqueous suspension (arrow shows the agglomeration) (the scale bar is 5 μm)

6.3.2.6 The effect of the Mw and number of hydroxyl groups on the size of the lignin microspheres

The particle size of ACL1-EA was significantly larger than other lignin acetate microspheres due to greater molecular weight of L1 than other lignins. The viscosity of the organic phase can be significantly increased by increasing the molecular weight of the polymer or the polymer concentration. When the viscosity of the organic phase is increased, it becomes complicated to form the microspheres from a viscous liquid.
(André-Abrant et al., 2001), and the particle size increases exponentially (Li et al., 2008). On the other hand, microspheres of L2 were successfully formed in all three cases (ACL2DCM, ACL2EA and L2EA) due to lowest molecular weight of L2 than other lignins. Therefore, it seems that molecular weight has an important role in the formation of the lignin microsphere by using emulsion solvent evaporation technique.

The hydroxyl groups of the lignin macromolecule demonstrated a significant effect on the microspheres formation. The microspheres were apparently formed in uniform shape and narrow size distribution when lignin acetate was used in the process. For instance ACL1DCM and ACL1EA were formed in a spherical shape, while L1EA was obtained with a high agglomeration and no uniform shape.

6.4 Conclusions

Synthesis of lignin microspheres through the solvent evaporation technique depends on two main factors; (1) the preparation parameters that are involved in the process and (2) the physico-chemical properties of lignin. The particle size of lignin microspheres was affected by altering preparation parameters: shear rate, mixing time, surfactant concentration and organic solvents that are essential factors in lignin microsphere formation. It was found that the particle size of lignin microspheres is decreased by increasing surfactant concentration, shear rate and agitation time. Uniform lignin acetate microspheres (with isolated lignins from different sources) were successfully prepared with an average size of about 1 μm by using either DCM or EA as organic solvents, homogenizer at 10,000 rpm for 30 seconds, and PVA as emulsifier with a concentration of 0.2 w/v%. ANOVA showed that the size of the lignin acetate microspheres that were prepared by using DCM remained in stable condition (p>0.05) over 35 days in a 0.1% neutral aqueous solution at room temperature, while particle size slightly increased within 10 days (p<0.05) when EA was used in the process. Lignin microspheres were successfully prepared with an average size of 1 μm without any pre-treatment (acetylation) from soluble parts of hardwood kraft lignin and non-wood soda lignin in EA.
CHAPTER 7 Final conclusions and future work

7.1 Summary and conclusions

This chapter is a summary of the main conclusions of this research which aimed to change the common view of the pulping industries and biorefineries. Biomass industrial processes are currently focused on profit from the cellulosic fractions, while this thesis proposes adding value to the lignin stream. This thesis discussed the entire process from the isolation of lignin from industrial residues to the value-added processes. Therefore, we propose the following steps: a comparison between the the physico-chemical and thermal characterization of lignin isolated from two main biomass industries, investigating the solubility of lignins in different organic solvents and finally synthesis of lignin microspheres. Two isolated lignins, L1 from bioethanol biorefinery residues and L2 from kraft black liquor, and two commercial lignins L3 and L4 were characterized to evaluate the potential of different lignins for value added products. The study concerning the physico-chemical characterization was focused on the properties of the lignins in order to identify the differences in functional groups and molecular weights. The molecular weight of L1 was high in comparison to the other lignins, while L2 had a very low molecular weight. Based on all results, L1 with the lowest hydroxyl number and highest Mw was closer to lignin in nature, while L2 with highest hydroxyl and lowest Mw was the most modified lignin. The relationship of the thermal properties and the impurities of lignin from different sources were investigated and it was observed that thermal properties of lignin strongly depended on the plant source and the extraction processes. Contaminations of the isolated lignins were mainly due to sugars and inorganic compounds. The thermal study showed that the onset decomposition temperature of all isolated lignins was almost similar to each other, while the glass transition value was significantly different. The solubility of lignin in organic solvents basically depended on the molecular weight and the number of hydroxyl groups. The solubility of acetylated lignin is increased in non-hydroxylated organic solvents due to replacement of the hydroxyl groups with acetyl groups. The solubility of lignin and lignin acetate in organic solvents is not completely predictable by using computed solubility
parameters. It has been found that the lignin acetate microspheres can be synthesized from different type of lignins through the emulsion solvent evaporation technique. The effect of the preparation parameters on the particle size of lignin microspheres through emulsion solvent evaporation was investigated in this thesis. This study also showed promising results for using EA as a less toxic solvent in the process of making lignin microspheres. For the first time, uniform lignin microspheres were synthesized from unmodified lignin isolated from kraft hardwood and non-wood soda lignins. The unique functionality of lignin microspheres (i.e. low cost, pH-sensitivity, biodegradability and sulfur-free in some cases) have great potential in pharmaceutical and agricultural industries.

7.2. Contributions

The objectives targeted in this thesis have been achieved through the four main studies and the following contributions have been made to the field of fabricating advanced materials (i.e. lignin microspheres) from lignin isolated from industrial by-products.

1. Physico-chemical behaviors and fundamental properties of lignins isolated from different sources (i.e. kraft, soda, and steam explosion) were evaluated and better understood. More importantly, suitable industrial applications were classified for different type of lignins based on their physico-chemical properties.

2. The effect of the impurities on the thermal properties of the bioethanol biorefinery residue and the kraft black liquor were determined and compared with each other.

3. Solubility of lignins isolated from different sources was determined in a series of organic solvents in order to find the relationship between the solubility of lignin and its physico-chemical properties. In addition, the solubility parameter of lignin was computed for prediction of the lignin solubility in organic solvents.
4. Synthesis of uniform lignin acetate microspheres from different industrial sources were developed by controlling the preparation parameters and using ethyl acetate as an alternative solvent.

5. A novel technique for synthesis of uniform lignin microspheres was proposed by using the soluble part of the industrial lignin in ethyl acetate and eliminating the pre-treatment (acetylation of lignin) through emulsion solvent evaporation technique. Lignin microspheres have great potential for advanced applications in pharmaceutical and agricultural industries.

7.3 Future work

In the future the following suggestions could be considered;

- Determination of the solubility of lignin isolated from different sources in a wide range of organic solvents in order to better understanding of the solubility of lignin in organic solvents.

- Development of lignin microspheres platform loaded with wide variety of chemical actives useful for agricultural and pharmaceutical applications.

- Lignin isolated from other industrial sources could be considered to obtain new lignin microspheres with unique properties.

- Scaling up the process of lignin microspheres production could be interesting.
References


Li, J., 2011. Isolation of lignin from wood, BSc thesis, Faculty of Technology. SAIMAA University of applied science, USA.


Telmo, C., Lousada, J., 2011. The explained variation by lignin and extractive contents on higher heating value of wood. biomass and bioenergy 35, 1663-1667.


Zabaleta, A.T., 2012. Lignin extraction, purification and depolymerization study
Department of Chemical Engineering and the Environment. Pais Vasco, Donostia-San Sebastián, p. 291.


