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DETERMINATION OF ASPEN LIGNIN

Master´s Thesis

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Table of Content

Table of Content 4
List of Tables 6
List of Figures 6
List of Abbreviations 10
ABSTRACT 11
INTRODUCTION 12
1. LITERATURE OVERVIEW 13
1.1 Wood 13
1.1.1 Cellulose 13
1.1.2 Hemicelluloses 14
1.1.3 Lignin 15
1.1.3.1 Chemical structure of lignin 15
1.1.3.2 Lignin in the cell wall 16
1.2 Wood pulp 18
1.3 Characteristic properties and uses of aspen wood and pulp 20
1.4 Residual lignin in BCTMP 21
1.5 Lignin determination 22
1.5.1 Lignin solubility 23
1.6 Lignin determination methods 23
1.6.1 Klason method 24
1.6.2 Staining reactions 24
1.6.3 Spectroscopy methods 26

1.6.3.1 Ultraviolet-Visible spectroscopy 27
1.6.3.2 Fourier transform infrared spectroscopy 31
2 EXPERIMENTAL SESSION 37
2.1 Materials 37
2.1.1 Aspen wood 37
2.1.2 Aspen wood pulp 37
2.1.3 Cellulose binder 37
2.2 Chemicals 37
2.2.1 Benzene 37
2.2.2 Sulphuric acid 37
2.2.3 Phloroglucinol 38
2.2.4 Hydrochloric acid 38
2.2.5 Ammonium hydroxide 38
2.2.6 Other chemicals 38
2.3 Apparatus 38
2.4 Preparation of samples 39
2.4.1 Aspen wood 39
2.4.2 BCTMP 40
2.5 Lignin determination 40
2.5.1 Determination of Acid Insoluble (Klason) Lignin 40
2.5.1.1 Isolation acid-insoluble lignin from aspen wood and BCTMP 41
2.5.2 FTIR spectroscopic experiments 41
2.5.3 UV spectroscopic experiments 42
2.5.1 Staining experiments 44
3 RESULTS AND DISCUSSION 46

3.1 Analysis of materials 46
3.1.1 Aspen wood and BCTMP 46
3.2 Klason lignin isolated from aspen wood and BCTMP 47
3.3 Staining reactions 48
3.3.1 Calibration of lignin for staining reactions 48
3.3.2 Weisner reaction 50
3.3.3 Mäule reaction 52
CONCLUSIONS 55
ACKNOWLEDGEMENTS 57
REFERENCES 58
Referaat 62

List of Tables

Table 1. Chemical comparison of various wood species (Sjöström 1993) 13
Table 2. Percent of intermonomeric linkage types in softwood and hardwood lignin (Sjöström, 1993). 16
Table 3. Functional groups of lignin (per 100 C_6C_3 Units) (Sjöström 1993). 16
Table 4. Content of the functional groups in the residual lignin of the aspen unbleached and bleached CTMP (mmol/g, o.d. lignin) (Fu and Qin 2013). 22
Table 5. Assignment of Main Infrared Spectral Bands for Lignin 35

List of Figures

Figure 1.1. The structure of cellulose (http://www.fibresource.com/f-tutor/cellulose.htm). 13
Figure 1.2. Chemical structure of mostly occurring monomers in hemicelluloses (http://www.rpi.edu/dept/chem-eng/Biotech-Environ/FUNDAMNT/hemicel.htm) 14

Figure 1.3. The basic units of lignin: (a) p-coumaryl, (b) coniferyl, and (c) sinapyl alcohol (Sjöström 1993; Sarkanen and Ludwing 1971). 15

Figure 1.4. Simplified structure of woody cell, showing the middle lamella (ML), the primary wall (P), the outer (S_1), middle (S_2) and inner (S_3) layers of secondary wall..... 17

Figure 1.5. Structure of the lignocellosics, lignin surrounds hemicelluloses and cellulose. (Figure adapted from Tomme et al., 1995). 17

Figure 1.6. Main process flow sheet for BCTMP (http://www.estoniancell.ee/en/1354/main-process-flow-sheet)..... 19

Figure 1.7. Micrograph of cross section of aspen wood..... 20

Figure 1.8. SEM images of BCTMP fibres. Middle lamella remainder can still be seen as in (a); some melted or partially melted lignin particles can be seen in (b) (Li and Tan 2006). 21

Figure 1.9. The electromagnetic spectrum (Owen 1996). 26

Figure 1.10. UV spectra of wood (a), lignin (b) and cellulose (c) (Mongeau and Brooks 2001). 28

Figure 1.11. Schematic of ultraviolet spectrophotometer..... 28

Figure 1.12. Calibration curves for the chosen wavelengths (212, 225, 237, 270, 280 and 287 nm) are shown for lignin extracted from aspen (Lee and Bédard 2013). 29

Figure 1.13. Reflection of light from the surface of solid sample. 30

Figure 1.14. UV spectra of MWL and dioxane lignin from Aspen (Jahan & Mun 2010). 31

Figure 1.15. IR spectra of cellulose, hemicelluloses and lignin of natural fibres (Liang and Marchessault 1959). 32

Figure 1.16. Operating principle of FTIR spectrometer (Griffiths & de Hasseth 2007). 33

Figure 1.17. A multiple reflection ATR system **(**http://www.utsc.utoronto.ca/~traceslab/ATR_FTIR.pdf). 34

Figure 2.1. The structure of benzene 37

Figure 2.2. The structure of phloroglucinol 38

Figure 2.3. Raw material: Aspen wood and aspen sawdust 39

Figure 2.4. Flask reactor with Reflux condenser 39

Figure 2.5. Photo of dried pulp mass from paper mill (left) and dried and grinded pulp (right). 40

Figure 2.6. Photo of Alpha FTIR spectrometer using platinum ATR 42

Figure 2.7. Photo of JASKO UV/VIV/NIR spectrophotometer V-670 (http://www.ttu.ee/keemia-ja-materjalitehnoloogia-teaduskond/materjaliteaduse-instituut/teadusaparatuur/) 43

Figure 2.8. Extractive-free aspen wood powder/microcrystalline cellulose binder were mixed (0%, 25%, 50%, 75%, 100% wt. % of aspen) and pressed into pellets for UV experiments..... 43

Figure 3.1. The IR spectra of aspen wood (violet) and BCTMP (red). 46

Figure 3.2. Photo of aspen wood lignin (left) and BCTMP lignin (right). 47

Figure 3.3. FTIR spectra of : 100% cellulose binder (red),25% wood + 75% cellulose binder (dark blue), 50% wood + 50% cellulose binder (blue), 75% wood + 25% cellulose binder (yellow), 100% wood (cyan). 48

Figure 3.4. Calibration curve for the detection of lignin content at maxima between 1234-1237 cm^{-1} for the comparative experiments of lignin colouring. 49

Figure 3.5. Pseudo-absorbance spectra of aspen wood powder/microcrystalline cellulose binder mixtures. Distinct absorbance maximum at 280nm was detected. 49

Figure 3.6. Calibration curve for the detection of lignin content at 280 nm for the comparative experiments of lignin colouring. 50

Figure 3.7. Photos show no colour difference between suspension samples of aspen wood(a) and BCTMP(b) stained with mixed phloroglucinol/HCl reagent before(left) applied to sample or added separately (right), reaction time 3 minutes. 50

Figure 3.8. Photos show the effect of different phloroglucinol/HCl ratio 2:1, 1:1, and 1:1 on suspension samples of aspen wood (c) and BCTMP (d) stained with phloroglucinol/HCl reagent, starting from top samples stained for 20 s, 2 min, 5 min and 20min. 51

Figure 3.9. Photos show different amount of aspen wood (e) and BCTMP(f) (from left 0,1g, 0,05g, 0,025g) suspension samples stained with phloroglucinol/HCl reagent, top without reagent HCl (e.1;f.1), bottom stained (e.2;f.2). 52

Figure 3.10. Photos show suspension of aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) samples stained with phloroglucinol/HCl 52

Figure 3.11. Photos show suspension of aspen wood (left) and BCTMP (right) samples treated with 1% KMnO4 solution for 2 minutes (g) and 10 minutes (h) 52

Figure 3.12 Photos show suspension of aspen wood (left) and BCTMP (right) samples treated with 3 % HCl solution for 20 s (a) and 2 minutes (b). 53

Figure 3.13. Photos show Mäule reaction´s third step - suspension containing samples with 1ml (a) and 2ml (b) NH_4OH solution. Reaction time: 1 minutes (k.1; l1), 5 minutes (k.2; l.2) and 10 minutes (k.3; l.3)..... 53

Figure 3.14. Photos of 1% KMnO4 filtering process 54

Figure 3.15. Photos show suspension of aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) samples treated with 1% KMnO4 solution (m) 3 % HCl solution (n) NH_4OH solution (o) 54

List of Abbreviations

BCTMP	bleached-chemi-thermo-mechanical pulp
TMP	thermo-mechanical pulp
CTMP	chemi-thermo-mechanical pulp
MP	mechanical pulp
PRC-APMP	preconditioning refiner chemical-alkaline peroxide mechanical pulp
NMR	nuclear magnetic resonance
FTIR	Fourier' transform infrared
SEM	scanning electron microscope
UV-VIS	ultraviolet-visible
NaOH	sodium hydroxide
$KMnO_4$	potassium permanganate
wt. %	weight percentage

ABSTRACT

Kärner, K. Determination of aspen lignin. Master's thesis. Tallinn University of Technology, Tallinn, 2014, 64 pages, 40 figures, 5 tables, in English.

LIGNIN, ASPEN WOOD, BCTMP, WEISNER REACTION, MÄULE REACTION, FTIR-ATR, UV-VIS

In the present study different methods for determination of aspen lignin were investigated. Dried samples of extractive-free aspen wood and grinded BCTMP pulp of aspen were used in experiments. For isolating lignin from aspen wood and BCTMP Klason method was used. For the quantification of lignin content a series of aspen wood powder/microcrystalline cellulose binder were mixed and analysed with FTIR-ATR and UV-VIS. For UV-VIS experiment samples were pressed into pellets. To compare with colour reactions an average content 21 % of lignin was assigned to experimental content in 100% aspen wood powder. FTIR-ATR absorbance maximum between 1234-1237 cm^{-1} and UV-VIS pseudo-absorbance of measured samples maximum at 280 nm, these maxima were taken as measurement points for the calibration of lignin content. Near linear dependence was established with both methods. Weisner and Mäule colour tests were used for staining reaction to detect lignin in samples. Suspension containing samples with staining solution were prepared and photographed. Best positive Weisner reaction with violet colour, both with aspen wood and BCTMP samples, were established with 1:1 phloroglucinol/HCl staining solution applied to 0.1 g sample and carefully mixed suspension, after 5 min reaction. With Mäule reaction intensive red colour to samples appeared after 10 min treatment with 1% $KMnO_4$ then washing with distilled water and treated with 3% aqueous HCl until the colour changes from black to beige/yellow, washed again and treated with concentrated NH_4OH for 2 minutes.

INTRODUCTION

Lignin is the third main component in wood, after cellulose and hemicelluloses. Normal hardwood contains between 20% and 30%, whereas normal softwood contains 26%–32% lignin. Due to the different types of linkages the structure of the lignin is rather complex and the composition of functional groups of lignin shows variations among the wood species. Therefore there is no generally applicable method for determination of lignin in lignocellulosics (Sjöström 1993; Adler 1977).

The presence and content of lignin in wood is determined by chemical, instrumental method or mixture of these methods which can be divided into direct and indirect method. A chemical method such as direct Klason method is the most common for lignin determination. In contrast to the direct determination of lignin content, indirect methods do not involve the isolation of a lignin residue. Staining reaction is one of the oldest and simplest methods for estimating the presence of lignin in wood. Spectroscopic methods (e.g. FTIR, UV-VIS) are often used for quantification and characterization of lignin.

For the rapid and routine detection of the presence of lignin there are techniques that may be applied to lignocellulosic materials *in situ*. For example staining reactions like Weisner and Mäule tests are widely used for identification of lignin in woody tissues.

The aim of the present thesis was to find effective and simple method for estimating the presence of aspen lignin and quantify it. Main focus was on staining reaction and finding the best methods and for identification of lignin in our samples. Klason method was used for isolating lignin from samples. Spectroscopic methods were used for quantification and for calibration of lignin for colouring reaction.

The experimental work was conducted in the Centre for Materials Research and in the Laboratory of Inorganic Materials at Tallinn University of Technology. Dry aspen wood and bleached-chemi-thermo-mechanical pulp (BCTMP) of aspen were used in the study. The present thesis is divided into three main parts. In the first part, literature overview of the topic is given. The second part of the thesis is experimental session, where the materials and methods used for experiments are described in detail and in the third part the results of the experiments are discussed.

1. LITERATURE OVERVIEW

1.1 Wood

Wood as one of the most abundant resources in the bio-based industry is basically composed of cellulose, hemicelluloses, lignin and extractives. Wood species can be divided into two groups: hardwood and softwood. The chemical composition of wood varies greatly between hardwoods and softwoods and also among tree species. **Table 1** shows some values that are given in the percentages of wood weight for each constituent in the different wood species (Sjöström 1993).

Table 1. Chemical comparison of various wood species (Sjöström 1993)

Constituent (%)	Softwood		Hardwood	
	Scots Pine (Pinus sylvestris)	Spruce (Picea glauca)	Silver Birch (Betula verrucosa)	Eucalyptus (Eucalyptus camaldulensis)
Cellulose	40.0	39.5	41.0	45.0
Hemicelluloses				
- Glucomannan (%)	16.0	17.2	2.3	3.1
- Glucuronoxylan (%)	8.9	10.4	27.5	14.1
- Other polysaccarides (%)	3.6	3.0	2.6	2.0
Lignin (%)	27.7	27.5	22	31.3
Total extractives (%)	3.5	2.1	3.0	2.8

1.1.1 Cellulose

Cellulose is a major component of wood, contributing 40-45% of the dry weight. It is assembled into a shell around the cell, thus forming the skeleton both for the cell and for the wood. Cellulose is a long-chain homopolysaccharide carbohydrate composed of β-glucose units attached together by β (1.4) linkages (**Figure 1.1**). Due to the strong hydrogen bonds that occur between cellulose chains, it has unique properties of mechanical strength and chemical stability. Cellulose chains consist of completely ordered regions or crystalline regions, which are changing into disordered or amorphous regions.

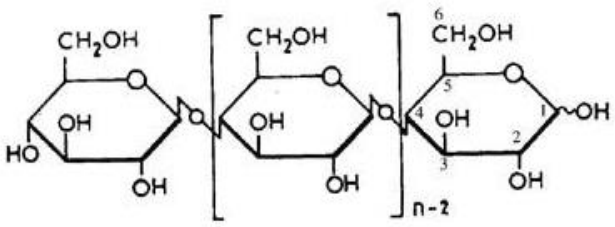


Figure 1.1. The structure of cellulose (http://www.fibresource.com/f-tutor/cellulose.htm).

Cellulose is the basic building block for paper and for many textiles. For example, cotton is composed of 87 -90% cellulose. In addition, cellulose can be used to make explosives and plastics. As cellulose is closely integrated with lignin and hemicelluloses, it is hard to separate cellulose from the wood in pure form (Sjöström 1993).

1.1.2 Hemicelluloses

Hemicelluloses are a group of amorphous polysaccharides. Hemicelluloses are heteropolymers composed of sugars like xylose, arabinose, glucose, galactose, mannose and sugar acids. These polymers contain most of the D-pentose sugars, and occasionally small amounts of L-sugars as well. Hemicelluloses are mostly straight-chained, but polymers with short side chains also occur. The main monomers in hemicelluloses are D-glucose, D-mannose, D-xylose, D-glucuronic acid and D-arabinose (**Figure 1.2**) (Scheller and Ulvskov 2010).

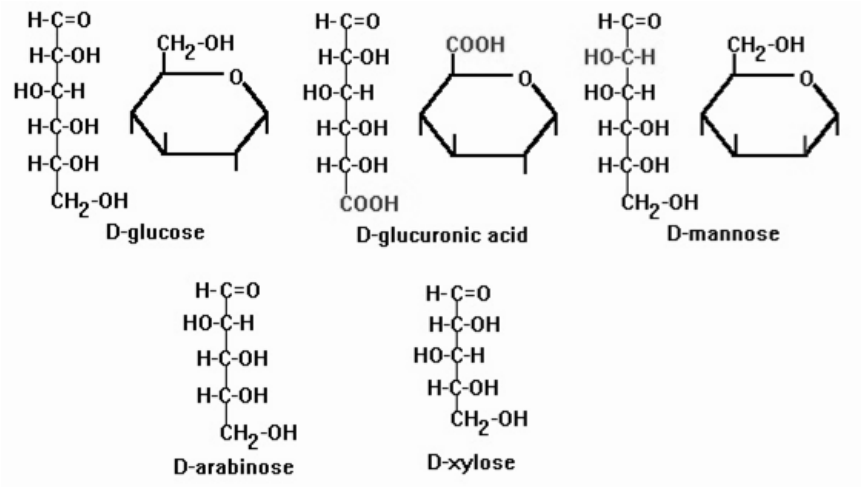


Figure 1.2. Chemical structure of mostly occurring monomers in hemicelluloses (http://www.rpi.edu/dept/chem-eng/Biotech-Environ/FUNDAMNT/hemicel.htm).

The amount of hemicelluloses of the dry weight wood is usually between 20 and 30%. The structure and composition of softwood and hardwood hemicelluloses are different. Hardwood hemicelluloses containing xylans, feature of a backbone ofβ-(1→4)-linked xylose residues whereas softwood consists of glucomannans, where the backbones are made of mannose and glucose. The most important role of hemicelluloses in the plant cell wall is their contribution to strengthening the cell wall by interaction with cellulose and lignin. Hemicelluloses have commercial significance, for example seed storage hemicelluloses are used directly as products in the food industry (Scheller and Ulvskov 2010).

1.1.3 Lignin

Lignin is an organic substance binding the cells, fibres and vessels which constitute wood. Normal hardwood contains between 20% and 30%, whereas normal softwood contains 26%–32% lignin. After cellulose, lignin is the second most abundant biological material (renewable carbon source) on Earth (Sjöström 1993).

1.1.3.1 Chemical structure of lignin

The chemical structure of lignin is rather complex, though composed of C, H, and O, lignin is not a carbohydrate but is phenolic in nature. The basic units of lignin are typically three phenylpropanoid monomers p-coumaryl, coniferyl and sinapyl alcohols (see **Figure 1.3**), also known as the H, G and S monolignols. Softwood lignin is composed mainly of p-coumaryl alcohol units. Guaiacyl lignin what is composed principally of coniferyl alcohol units, is found in softwoods. Guaiacyl-syringyl lignin contains monomeric units from coniferyl and sinapyl alcohol, is present in hardwoods (Sjöström 1993; Sarkanen and Ludwing 1971).

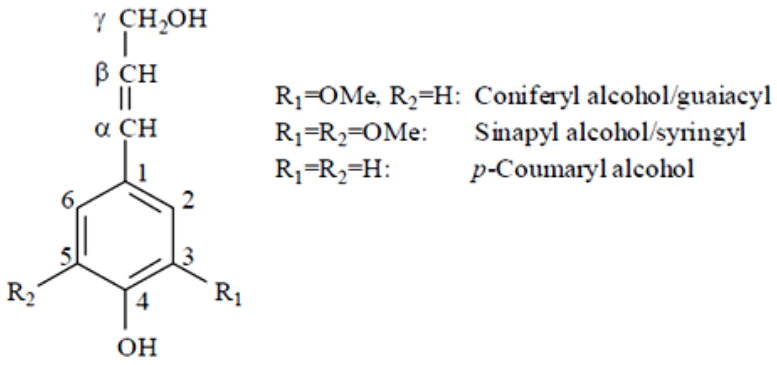


Figure 1.3. The basic units of lignin: (a) p-coumaryl, (b) coniferyl, and (c) sinapyl alcohol (Sjöström 1993; Sarkanen and Ludwing 1971).

Lignin consists of 15 to 18 phenylpropane units, which are joined together both with C–O–C ether and C–C carbon–carbon linkages. The position of a linkage may be at any of several locations on each phenolic unit, causing many different linkage types to be possible. The most common linkage types found in a lignin molecule are β-O-4, α-O-4, β-5, 5-5, 4-O-5, β-1, and β-β (**Table 2**) (Dence 1992; Sjöström 1993).

Table 2. Percent of intermonomeric linkage types in softwood and hardwood lignin (Sjöström, 1993).

Linkage Types	Percent of total linkages	
	Softwood Lignin	Hardwood Lignin
B	50	60
α-O-4	2 – 8	7
β-5	9 – 12	6
5-5	10 – 11	5
4-O-5	4	7
β-1	7	7
β-β	2	3

Lignin polymer contains different functional groups - methoxyl, phenolic hydroxyl, benzyl alcohol and carbonyl groups and some terminal aldehyde groups in the side chain. There is considerable variation in the composition of functional groups among different wood species and also within the cell walls and in many cases the exact structure of lignin is unknown. Approximate values for the frequencies of different functional groups can be given (**Table 3**) (Sjöström 1993).

Table 3. Functional groups of lignin (per 100 C_6C_3 Units) (Sjöström 1993).

Group	Softwood Lignin	Hardwood Lignin
Methoxyl	92-97	139-158
Phenolic hydroxy	15-30	10-15
Benzyl alcohol	30-40	40-50
Carbonyl	10-15	

1.1.3.2 Lignin in the cell wall

Lignin is the third main component in wood, after cellulose and hemicelluloses. It occurs between and within cell walls. The cell wall consists of three main layers: middle lamella, primary wall and secondary wall (**Figure 1.4**). Lignification occurs first along the middle lamella and extends thereafter, through the primary wall into the secondary wall. The tangential walls are usually lignified somewhat before the radial walls. Although the highest concentration of lignin (about 60%) is found in the middle lamella, but, because the layer is thin, only a minor fraction of the total lignin is located in this layer. The majority of lignin is contained the S_2 layer, evenly distributed throughout the cell wall, but in lower concentrations (Sjöström 1993).

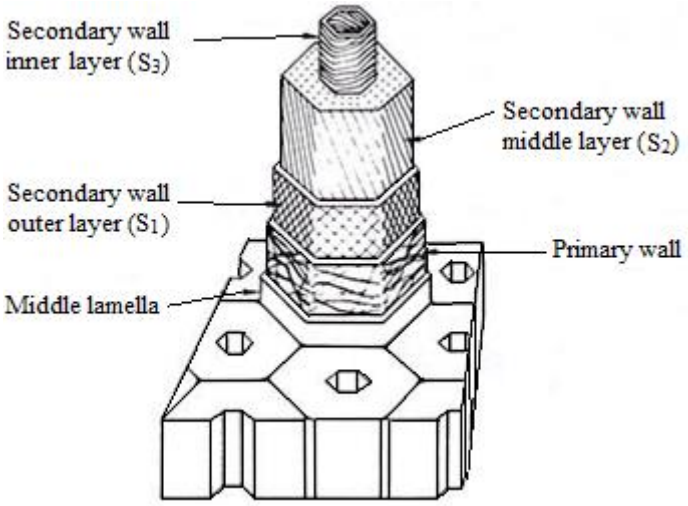


Figure 1.4. Simplified structure of woody cell, showing the middle lamella (ML), the primary wall (P), the outer (S_1), middle (S_2) and inner (S_3) layers of secondary wall.

Lignin surrounds the preformed hemicelluloses and cellulose to form a strong and hydrophobic secondary wall called lignocellulosics (**Figure 1.5**). Between cell walls lignin acts as a binding agent holding cells together and within cell wall it gives rigidity to the cell.

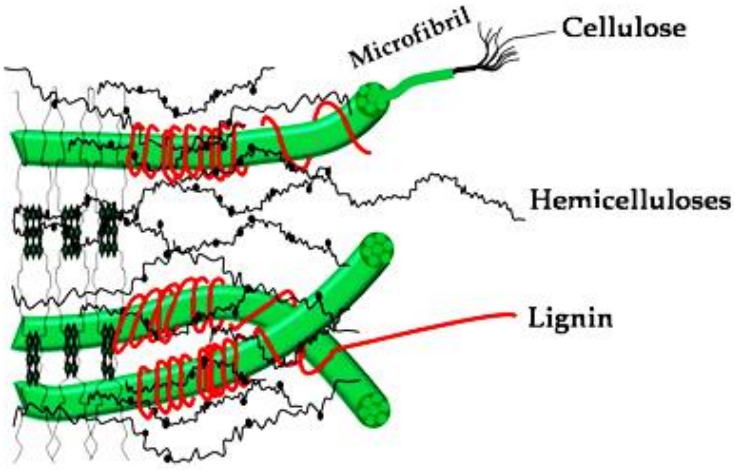


Figure 1.5. Structure of the lignocellosics, lignin surrounds hemicelluloses and cellulose. (Figure adapted from Tomme et al., 1995).

Lignin also decreases the permeation of water across cell walls in conducting with the xylem, thereby playing an intricate role in the transport of water and nutrients. Finally, lignin adds to wood's toxicity by impeding penetration of destructive enzymes through the cell wall, making wood resistant to decay and insect attack. Although lignin is necessary to trees, it is undesirable in most cellulose fibres used and is mainly removed by pulping and bleaching processes (Sjöström 1993; Sarkanen and Ludwing 1971).

1.2 Wood pulp

Chinese were the first to invent processes for paper manufacture in 105 AD, paper remained a luxury item through the centuries, as printing and education increased the demand for paper increased development of a number of methods for producing various grades of pulp. Starting from the 19th century paper can be made with fibres from wood pulp using steam-driven papermaking machines. Nowadays wood pulp is also used mainly for papermaking, but in addition is found use in textiles, plastics and explosives industries (Asunción 2003).

During pulping processes, the purpose of which is to separate cellulose fibres from solid wood, hemicelluloses are completely or partially degraded together with the lignin matrix, depending on the pulping process used. The main aim is to remove as much of the lignin as completely as possible whilst keeping the cellulose and hemicelluloses intact to increase the yield of the fibres. For extraction of pulp fibres from wood four general methods are used - chemical, mechanical, thermo- and chemi-mechanical processes (Sjöström 1993; Eklund and Lindström 1991).

Mechanical pulping applies mechanical force on wood by pressing block of wood against a grinding stone to separate the fibres. There are two main processes used for the manufacturing of mechanical pulping. In the stone ground wood process (SGW) logs are pressed against a rotating grinder stone with simultaneous addition of water. Thermo-mechanical pulp (TMP) is when wood chips are first softened with intense heat and immediately followed by applying a mechanical force to grind heated wood chips into pulp. In the mechanical pulping, the objective is to maintain the main part of the lignin in order to achieve high yield with acceptable strength properties (Ek 2009).

In the chemi-thermo-mechanical-pulping process (CTMP) the wood chips are pre-softened before defibrillation at rather mild conditions with chemicals. CTMP is generally considered a mechanical pulping technique since the chemicals principally soften the lignin prior to the mechanical stage rather than fully dissolve it out as in true chemical pulping processes. By this treatment, sulfonic acid groups are introduced into the lignin, making it more hydrophilic and increasing the degree of swelling of the pulp (Eklund and Lindström 1991; Sjöström 1993).

All pulps require bleaching in order to remove residual lignin and achieve brightness. There are many different types of bleaching processes involving different chemicals and conditions. Bleaching chemicals are oxidizing agents that break up the lignin molecule, introduce solubilizing groups into the fragments, disrupt lignin-carbohydrate bonds, allowing fragments to dissolve (Ek 2009).

The BCTMP (bleached chemi-thermomechanical pulp) process is a mechanical pulping technology which uses sodium sulfite for impregnation. The BCTMP technology is non lignin destructive bleaching used to retain yield for both softwood and hardwood prior to the first refining stage. The fibres are bleached in a conventional peroxide or ozone bleach plant after refining and screening. Aspen BCTMP is produced using the PRC-APMP (Preconditioning Refiner Chemical - Alkaline Peroxide Mechanical Pulp) process combines the concept of applying chemicals such as alkaline peroxide pre-treatment to lignocellulosic material before primary refining with the concept of applying chemicals such as alkaline peroxide at the primary refiner. Main process flow sheet of BCTMP can be seen in **Figure 1.6**.

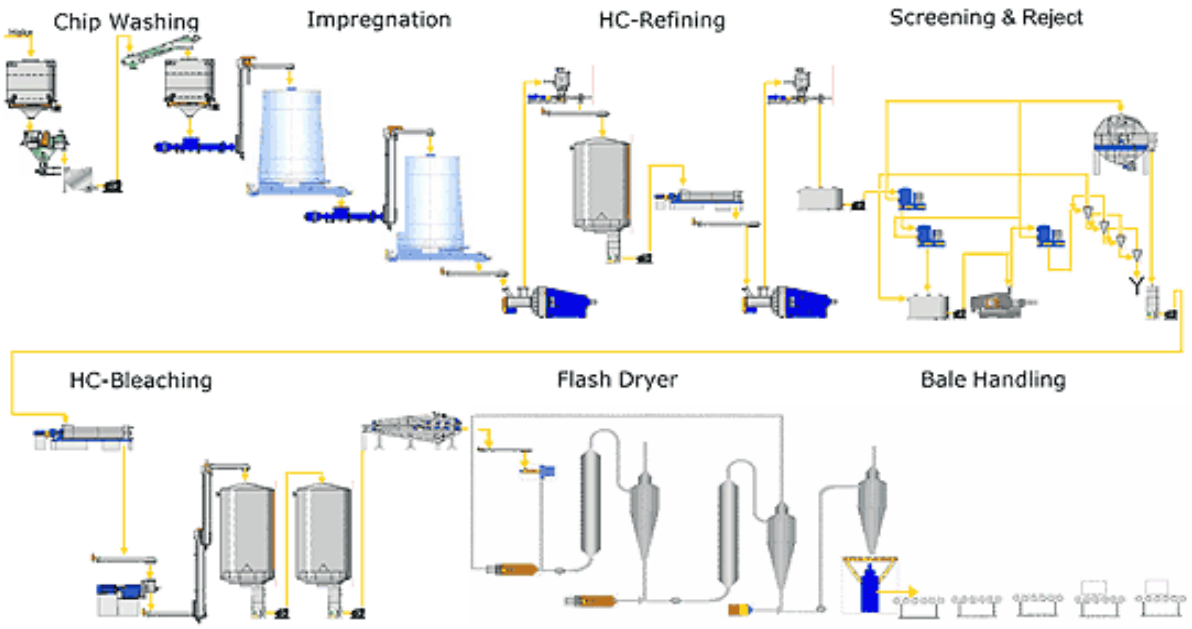


Figure 1.6. Main process flow sheet for BCTMP (http://www.estoniancell.ee/en/1354/main-process-flow-sheet)

1.3 Characteristic properties and uses of aspen wood and pulp

Aspen is a diffuse-porous hardwood. The pores are small and evenly distributed throughout annual growth increments (**Figure 1.7**).There are 26% of vessel elements and 61% of wood fibres in aspen wood. Fibre length is 0.7-1.6 mm and width is 0.01-0.03 mm, the thickness of cell wall is 0.02-0.007 mm. There is approximately 48% of cellulose and 21% of lignin in aspen wood. Aspen wood has low flammability therefore it is used for making matches and paper, where makes it safer to use than most other woods. Due to relative ease of aspen machining and gluing it is also used for products like furniture components, toys, containers (Pickering2008; Lamb 1967).

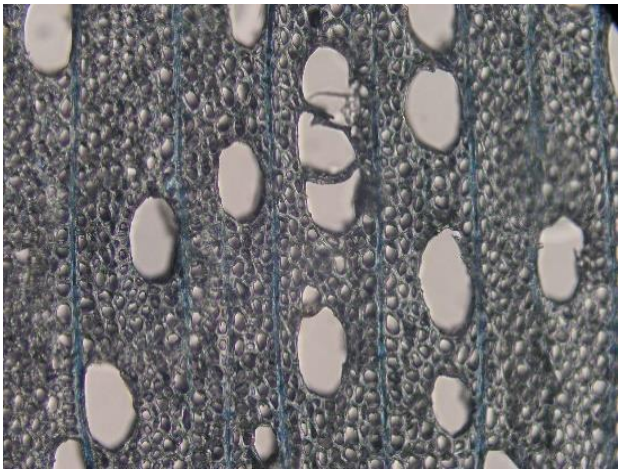


Figure 1.7. Micrograph of cross section of aspen wood

Due to fibre morphology, aspen wood has excellent length-to-diameter ratio and fibre wall thickness. It is characterized as thin to medium therefore is easily pulped by all commercial processes. Aspen pulp is mainly used to produce book, newsprint, and fine printing papers. The highest quality groundwood pulps are produced from aspen. Aspen pulp is mainly produced from kraft and sulfite pulps. Typical properties of aspen kraft pulp are – fibre length 1.0-1-3 mm, width 18-19 μm, wall thickness 2.0-3.0 μm and fibre coarseness 86 μ g/m. Chemi-mechanical pulps produced from aspen are used primarily for hardboards and fibreboards. Aspen bleached chemi-thermomechanical pulp contains 17% lignin which is relatively high quantity compared to other bleached pulps. Despite that it has high brightness and low coarseness at high freeness levels what makes it ideally suited for use in traditional wood free applications. Due to its relatively short fibre length (about 0.8mm) and high fines content (17% to 25%), aspen BCTMP offers superior formation compared to most kraft pulps (Reis and Nielsen 2001).

1.4 Residual lignin in BCTMP

Some studies have shown that mechanical pulping and bleaching does not remove all reminders of middle lamella. Li and Tan (Li and Tan 2006) suggested that if in the mechanical pulping process the fibre separation takes place in the middle lamella region, middle lamella material will most likely attach to or remain on the surface of the adjacent fibres. Because middle lamella has about 60% lignin, the fibre surface will definitely contribute to high surface lignin concentration. They found that residual lignin can be seen on even after bleaching on BCTMP fibres. The lignin present on the BCTMP fibre will affect the mechanical property and the inter-fibre bonding ability of individual fibres. SEM images of BCTMP fibres with middle lamella reminder are presented below in **Figure 1.8** (Sjöström 1993).

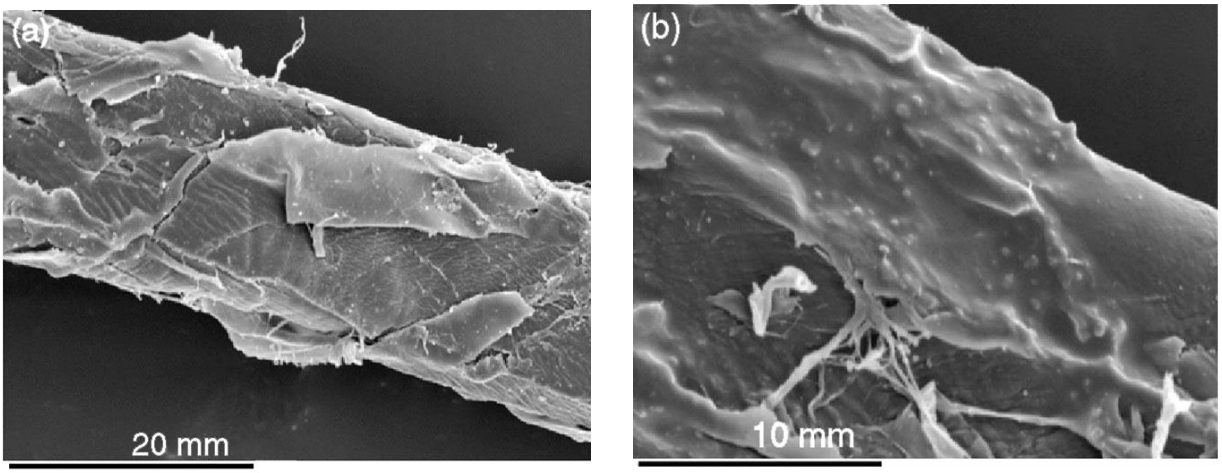


Figure 1.8. SEM images of BCTMP fibres. Middle lamella remainder can still be seen as in (a); some melted or partially melted lignin particles can be seen in (b) (Li and Tan 2006).

Recently Fu and Qin investigated aspen alkaline peroxide-impregnated chemi-thermomechanical pulp fibres during alkaline peroxide bleaching and performed quantitative evaluation of functional groups in residual lignin of the aspen unbleached and beached CTMP by 31P-NMR (Phosphorus-31 nuclear magnetic resonance spectroscopy) (**Table 4**). They found that new carboxyl groups were introduced into lignin structure and alkaline peroxide bleaching reduced the contents of total phenolic –OH and various kinds of phenolic –OH in residual lignin with increasing dosages of NaOH and H_2O_2 (Fu and Qin 2013).

Table 4. Content of the functional groups in the residual lignin of the aspen unbleached and bleached CTMP (mmol/g, o.d. lignin) (Fu and Qin 2013).

Pulps	Aliphatic -OH	Condensed phenolic -OH	Guaiacyl and demetylated -OH	Syringyl -OH	p-hydroxyl -OH	Total phenolic -OH	Carboxylic acid
Unbleached	4.50	0.28	0.27	0.52	0.46	1.53	0.18
1% H_2O_2 / 2% NaOH	5.32	0.25	0.23	0.40	0.34	1.22	0.31
3% H_2O_2 / 2% NaOH	7.95	0.17	0.22	0.35	0.25	0.99	0.41
3% H_2O_2 / 4% NaOH	5.11	0.15	0.21	0.32	0.30	0.98	0.25

The structural details of residual lignin are of considerable importance in pulp delignification. In addition, the reactions taking place during different chemical treatments can be characterized by the changes in residual lignin structure (Chang 1992).

1.5 Lignin determination

The determination of lignin is an investigation of lignocellulosic material, performed for characterizing and for evaluating the effects of different treatments (chemical, mechanical, and biological) of wood and pulp. In addition, determination of lignin is used for observing effluents in wood processing industries and for estimating bleach chemical requirements (Dence 1992).

A number of spectral methods for determining lignin content are based on totally dissolving the sample in a suitable solvent and measuring the absorbance of the solution. These methods are dependent upon the specificity with which lignin is hydrolysed, as well as the yield of solubilized lignin. The experimental difficulty in studying the macromolecular properties of lignin is the fact that lignin has a very low solubility in most solvents. Furthermore, lignin behaves quite differently in solution in comparison with cellulose (Horvath 2006).

1.5.1 Lignin solubility

The solubility behaviour of lignin is that which should be expected of a polar aromatic polymer with many hydroxyl groups. Lignin fragments of lower molecular weight are more soluble in a wide range of solvents than higher molecular weight components. The solubility of lignin is greater with hydroxylated solvents, e.g., methanol, ethanol, phenol, and water. Although native lignin behaves as an insoluble, isolated lignin exhibit maximum solubility in solvents including, for example, dioxane, acetone, methyl. Other polymers like natural rubber, PVC, polystyrene, and polyisobutylene show similar behaviour to lignin (Horvath 2006).

Lignin fractions are most soluble in solvents with solubility parameters of around 11 $(cal/cc)^{1/2}$. The hydrogen-bonding capacities of various solvents are proportional to the shift in wave length of the infrared region of the spectrum. Schuerch tested several solvents and found that lignin solvents with solubility parameters around 11, for example acetone, dioxane, pyridine, and glycol ethers shift in wave length values of 0.14 μm, and named them good lignin solvents (Schuerch 1952).

The solubility of lignin is greater in the mixture than in either solvent alone. The hydrogen-bonding capacity of the mixture is greater than that of one individual solvent and often the solubility parameter of the mixture is higher than the pure solvent. The mixture of alcohol with dioxane is a more effective solvent than individual solvents (Schuerch 1950, 1952).

Residual lignin in bleachable grade pulps is almost completely soluble in dioxane, dimethylformamide, dimethylsulfoxide and 1M NaOH.

1.6 Lignin determination methods

There are several standard methods but no generally applicable method for determination of the total amount of lignin in wood and pulp samples. Lignin content in wood can be determined by using direct or indirect methods. The direct methods involve the isolation of a lignin from wood or pulp such as Klason method which includes measurement of acid-insoluble lignin. Indirect methods include mainly instrumental such as spectrophotometric methods, but also chemical method using staining reactions for lignin detection, used typically for wood pulps. Spectroscopic methods are generally considered the best basis for identification of lignin. Klason method, staining reactions and spectroscopic methods such as UV- and FTIR-spectroscopy for lignin determination are described below (Hatfield and Fukushima 2005).

1.6.1 Klason method

Klason method is probably the simplest and overall the most reliable therefore the most widely used for lignin determination, despite its limitations. The standard Klason lignin protocol has been well covered by Dence. Method is based on digestion of samples with 72% sulphuric acid, then with dilute sulphuric acid, to hydrolyse and solubilize the polysaccharides; the insoluble residue is dried and weighed as lignin (Browning 1967; Dence 1992).

The major disadvantages of the procedure are as follows: other components, including proteins and suberins, may condense and analyse as Klason lignin and some lignin, notably those highly enriched for syringylpropane units are partially solubilized. The lignin remaining in solution after the sulphuric acid has been diluted may represent as much as 3-5% of the total lignin (in angiosperms). Given the variability in syringyl lignin content across various species of angiosperms, a single version of the Klason procedure cannot be generalized across all angiosperms (Dean1997).

1.6.2 Staining reactions

Dyes to make structures within sections of plant material clearly visible, has been used since 1900. Perhaps one of the oldest and simplest methods to show the presence or absence of lignin within the fibre cell walls is the use of staining agents. Method is based on characteristic colour reactions between lignified plant tissues with numerous organic and inorganic reagents and then lignin is examined at the macroscopic or optical microscopy levels. (Gray 1971; Christiernin and Ohlsson 2005).

Certain stains and colour reactions like Weisner and Mäule reaction have emerged as the most widely used diagnostic tests for lignin. The coniferyl and sinapyl aldehyde groups of lignin appear to react with phloroglucinol-HCl (the Weisner reaction) to give a red-violet colour. These reactive groups are present in only small quantities in lignin, but the test is quite sensitive and because of the ease of staining, this procedure is still often used as one of the tests for identification the presence of lignin in plant cell wall. The test should be used carefully because colouration may be weak or even absent in lignin containing high amounts of syringyl units (Sarkanen and Ludwing 1971; Harkin 1966; Dence 1992).

In Mäule test lignin reacts differently in hardwoods and softwoods, therefore lignin can be differentiated using this reaction. In this test, sequential wood treatments with potassium permanganate and hydrochloric acid convert guaiacyl and syringyl residues to catechol and methoxycatechol moieties, respectively. Subsequent treatment with concentrated ammonium hydroxide gives a red colour if derived from a hardwood species, but only a dirty brown colour if derived from a softwood species. The red colour is due to the higher content of units derived from sinapyl alcohol in hardwoods (Harkin 1966; Gray 1971).

The preceding methods are convenient for studying lignin at the macroscopic or optical microscopy levels. One method is to take a photo of lignin-containing suspension; this method is invaluable if the aim is to determine the nature of living plant cells, but can be also used for dection of lignin in dead cells of wood and pulps. Recently Christiernin and Ohlsson used this method to show presence of lignin in the hybrid aspen cell cultures cultivated up to 21 days (Christiernin and Ohlsson 2005).

Optical microscopy refers to the inspection of the sample at higher magnification using visible light. During light microscope inspection, the specimen is positioned perpendicularly to the axis of the objective lens. Light is then shown on the sample, which reflects some light back to the lens. For investigation wood cells with optical microscope most specimens are fixed on glass slides. The image seen in the microscope depends not only on how the specimen is illuminated and positioned, but on the characteristics of the specimen as well (Schade 2001).

Discretion must be used with these reactions because almost all the components of wood will produce coloured products upon application of selected reagents, therefore sometimes it is necessary to remove one or more of wood constituents (polysaccharides and extractives) in order to accurately detect lignin or to use multiple tests. Another important thing to take into consideration is that the chemical nature of a particular constituent within the cell wall may be quite different than its nature after removal from the wall. Also different treatments can affect lignin colour reaction and a negative reaction does not necessarily mean that lignin is absent; therefore it is advisable to use several methods on the same material in order to be reasonably sure of detecting lignin (Browning 1967; Gray 1971).

1.6.3 Spectroscopy methods

Ultraviolet-Visible (UV-VIS) and infrared (IR) are part of the electromagnetic spectrum, which includes such other forms of radiation as radio, cosmic, and X rays (see **Figure 1.9**).Molecular spectroscopy involves the absorption of electromagnetic radiation by the material whose molecular structure we are attempting to determine (Owen 1996).

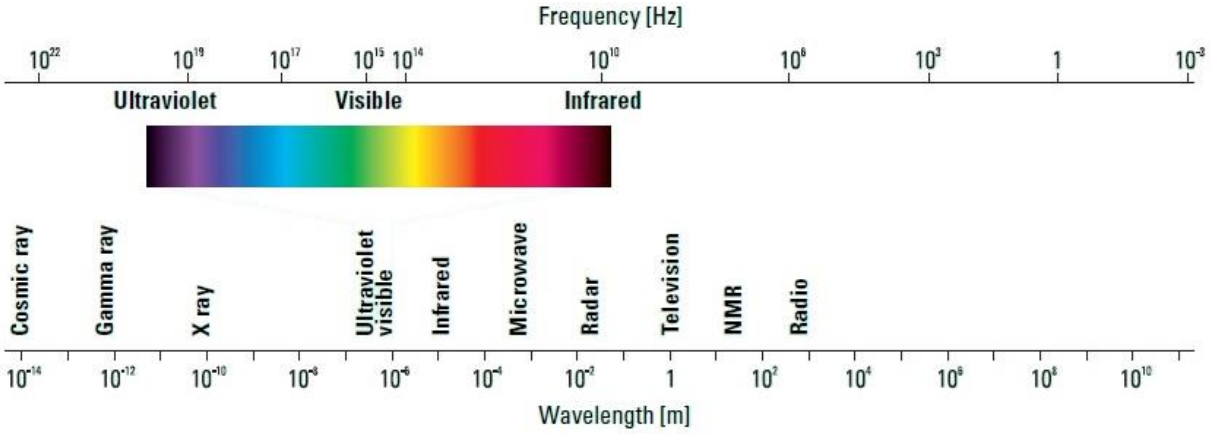


Figure 1.9. The electromagnetic spectrum (Owen 1996).

Electromagnetic radiation can be characterized by its energy E (J), wavelength λ (μm), frequency ν (Hz) or wavenumber $\tilde{\nu}$ (cm^{-1}) – the number of waves per centimetre. There are related to each other through (Owen 1996):

$$\boldsymbol{E = h\nu = \frac{hc}{\lambda}} \qquad [1]$$

Where:

E is energy (in joules),

h is Planck's constant (6.62 × 10-34 Js),

ν is frequency (in seconds),

c is the velocity of the radiation in vacuum (2.9979·108 m/s)

When light passes through or is reflected from a sample, the amount of light absorbed is the difference between the incident radiation (Io) and the transmitted radiation (I). The amount of light absorbed is expressed as either transmittance (T) or absorbance (A).

Evaluation expressed as absorbance (Smith 2011):

$$\boldsymbol{A_\lambda = \log \frac{I_0}{I}} \qquad [2]$$

Absorbance is also related to concentration of molecules in the sample, the relationship is described by Beer`s law. The absorption (A) is proportional to the inherent absorbing ability of the substance ε (molar absorptivity or extinction coefficient), the concentration of the absorbing compound (c) and the distance the radiation is travelling through the sample (b).

Beer's law can be expressed as (Smith 2011):

$$\boldsymbol{A_{\lambda} = \varepsilon bc} \qquad [3]$$

Where:

A_{λ} = average absorbance at a specific wavelength,

ε = absorptivity constant at a specific wavelength in L/g-cm

b is the path length through the sample in cm,

c is the concentration of a single analyte in mg/ml.

When radiation passes through a sample, part of the radiation may be absorbed by the sample provided that there is a change in the dipole moment during the vibration. The energy gained by a molecule in this way may bring about increased vibration or rotation of the atoms, or may raise electrons to higher energy levels. The particular frequency of radiation that a given molecule can absorb depends upon the changes in vibrations or rotations or electronic states that are permitted to a molecule of that structure.

1.6.3.1 Ultraviolet-Visible spectroscopy

Since lignin is the predominant UV-absorbing material in wood (**Figure 1.10**), UV spectrophotometry is an appealing and simple method for lignin quantification and has been used for the determination of lignin in wood. When the intensity of absorption is plotted against a given wavelength of ultraviolet light, an ultraviolet spectrum curve for the lignin is obtained. According to the type of lignin, the lignin solvent, and the pH of the solution and lignin structure, the shape of this curve may change (Mongeau and Brooks 2001; Glennie and McCarthy 1962).

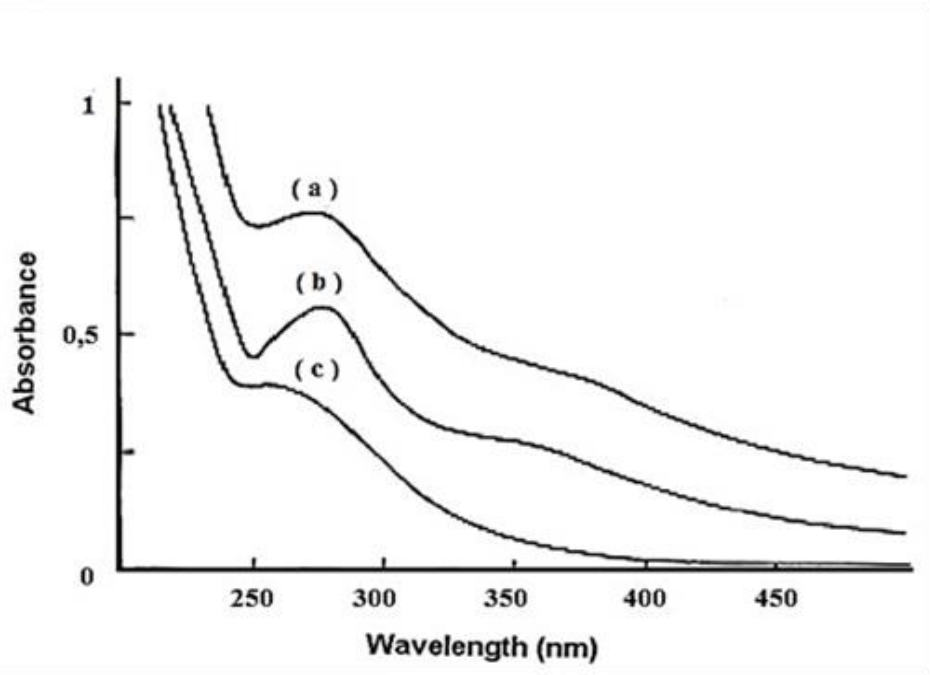


Figure 1.10. UV spectra of wood (a), lignin (b) and cellulose (c) (Mongeau and Brooks 2001).

Basically there are two kinds of UV spectrophotometers, single-beam and dual-beam. Both of these spectrometers consist of light source, filter of monochromator, sample and detector (**Figure 1.11.**). In single-beam UV spectrophotometer the absorbance of a sample is determined by measuring the intensity of light reaching the detector without the sample (the blank) at first and then comparing it with the intensity of light reaching the detector after passing through the sample. Dual-beam spectrophotometer enables the blank and the sample to be measured at the same time. Ideally the blank should consist of same components as sample without (Owen 1996).

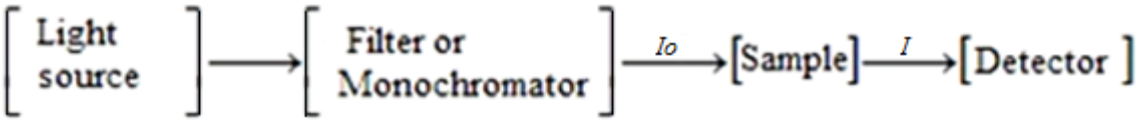


Figure 1.11. Schematic of ultraviolet spectrophotometer

Different matters absorb light at different wavelengths to varying degrees, therefore the amount of light absorbed can be used for quantification and the shape of spectra basically for identification the components in the sample. The concentration of unknown sample can be determined using Beer´s Law and dilution factor relative to the intact sample.

In the qualitative and quantitative UV spectroscopic determination of lignin the typical maximum at a wavelength of 280 nm which indicates to presence of benzene ring in lignin structure, is mostly used. Because the lignin molecule contains no large portion of unsaturated aliphatic units in addition to its aromatic structure, it is concluded that there are the two characteristic bands in the lignin spectrum at 200-230 and 260-280 nm. As lignin is photosensitive, it is essential to coat each vial containing lignin with aluminium until ready to be analysed with the UV/VIS (Hon and Shiraishi 2000).

Recently Lee and Bédard used UV–VIS as quantification tool for solubilized lignin following a single-shot steam process. They produced lignin from different types of biomasses including aspen using steam processes. For lignin calibration and concentration determination they obtained adsorption maxima at 212, 225, 237, 270, 280 and 287 nm. All standard samples were dissolved in 0.035 M NaOH and reference solution was consistent with NaOH solutions used for the samples. Their results showed that the concentration of aqueous lignin can rapidly be tested, with little or no pre-treatment and with a limited usage of chemicals. They established significant correlation between the adsorption and lignin concentrations between 0.005 and 0.12 g/L of lignin in 0.035 M NaOH. Calibration curves for the chosen wavelengths for lignin extracted from aspen are shown in **Figure 1.12**. (Lee and Bédard 2013).

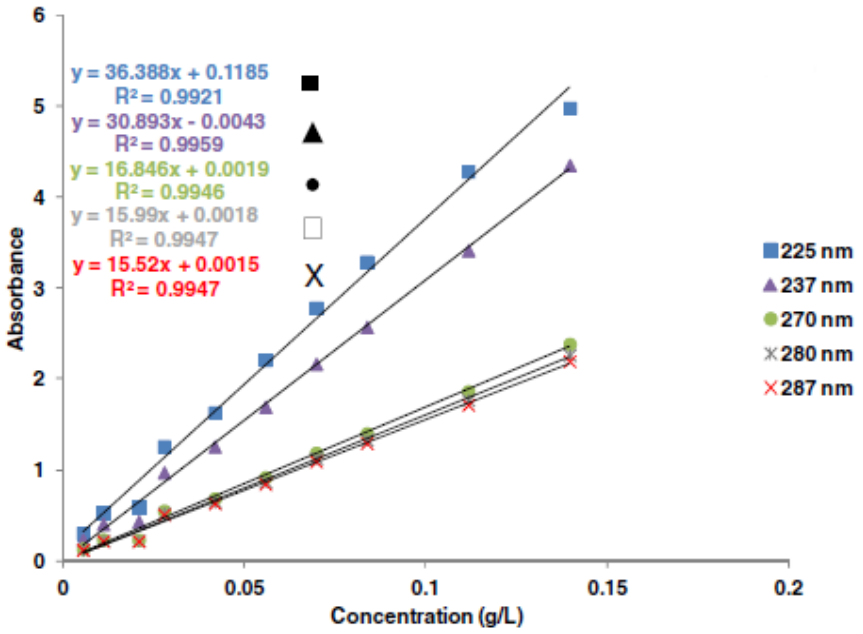


Figure 1.12. Calibration curves for the chosen wavelengths (212, 225, 237, 270, 280 and 287 nm) are shown for lignin extracted from aspen (Lee and Bédard 2013).

The diffuse reflectance is proper sampling tool for solid materials in UV-VIS spectral ranges. It has advantages like easy preparation of samples compared to the transmission analysis. When the spectrometer beam is focussed onto the solid sample surface, part of that is reflected, scattered and transmitted through the sample material (**Figure 1.13.**). The back reflected, diffusely scattered light is collected by the accessory and directed to the detection optics. Diffuse reflection of the beam is only that part what is scattered within the sample and returned to the surface.

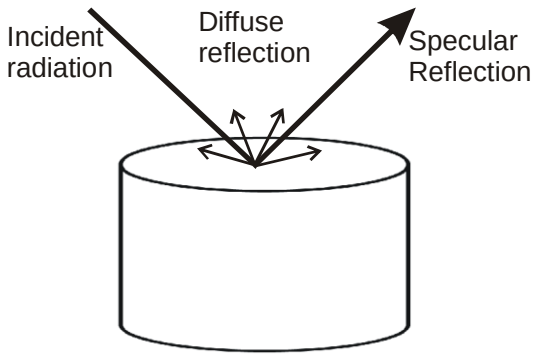


Figure 1.13. Reflection of light from the surface of solid sample.

The raw diffuse reflectance spectra appear different from its transmission equivalent. Therefore a Kubelka-Munk conversion is applied to diffuse reflectance spectrum to compensate for these differences and pseudo-absorbance spectra are created. Theoretically the Kubelka-Munk equation creates linear relationship for spectral intensity relative to sample concentration in "infinitely thick" sample layer (Kubelka 1948).

The Kubelka-Munk equation is expressed as follows (Kubelka 1948):

$$f(\boldsymbol{R}) = \frac{(\mathbf{1}-\boldsymbol{R})^{\mathbf{2}}}{\mathbf{2}\boldsymbol{R}} = \frac{\boldsymbol{k}}{\boldsymbol{s}} \qquad [4]$$

Where:

R is the absolute reflectance of the sampled layer;

k is the molar absorption coefficient;

s is the scattering coefficient.

Diffuse reflectance spectroscopy has proven to be a fast and sensitive technique for qualitative information; however quantitative data can be also derived. Samples prepared for diffuse reflectance measurements should be uniformly and well mixed. Non-homogenous samples lack reproducibility and will be difficult to quantify (Péré and Cardy 2000).

Different treatments of wood may have effect on lignin UV spectra. Jahan and Mun extracted lignin from finely ball-milled wood with aqueous dioxane which is the most used techniques of isolating lignin from wood in a chemically unaltered form then investigated with UV spectroscopy. Aspen lignin had maximum at 274 nm in both MWL (milled wood lignin) and dioxane lignin (**Figure 1.14**). This fact contributes to the higher symmetry of the phenylpropane units in aspen lignin caused by the higher syringyl units. It is known that guaiacyl compounds exhibit maximum in the region of 280 nm (Jahan & Mun 2010).

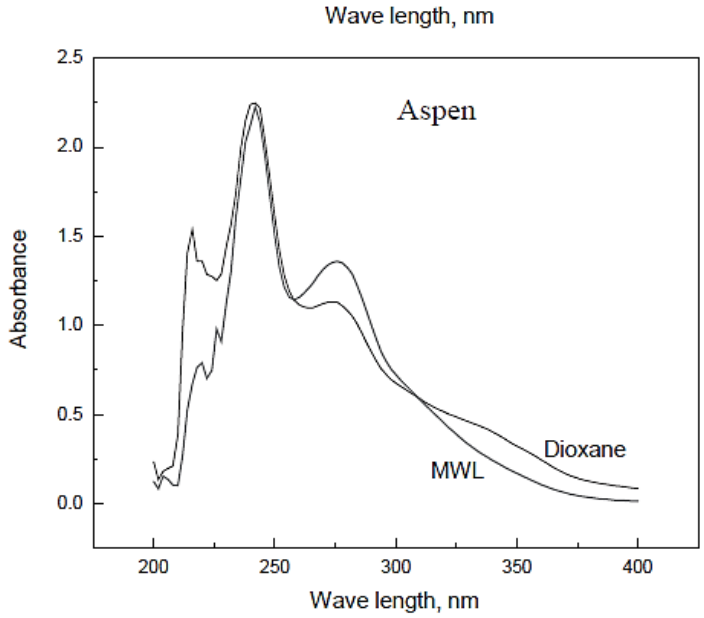


Figure 1.14. UV spectra of MWL and dioxane lignin from Aspen (Jahan & Mun 2010).

1.6.3.2 Fourier transform infrared spectroscopy

Since lignin absorbs relatively strongly in the IR regions compared with cellulose or hemicelluloses (**Figure 1.15**) infrared spectroscopy has become a preferred technique for rapid *in situ* analysis widely used for both characterization and qualification of lignin. FTIR spectroscopy is valuable for analysing the chemical structure of lignin: lignin type (the phydroxyphenyl, guaiacy and syringyl units), methoxyl groups, carbonyl groups, and the ratio of phenolic hydroxyl to aliphatic hydroxyl groups (Heitner and Dimmel 2010; Faix 1992).

Infrared spectra of lignin were investigated and partially interpreted in 1940's through 1950's In the past, spectra were recorded using the so-called dispersive technique, i.e., with grating-type or prism instruments. Since the early 1980's Fourier transform infrared (FTIR) spectrometers have become increasingly available for routine laboratory work (Jones 1948; Buchanan and Brauns 1949; Liang and Marchessault 1959).

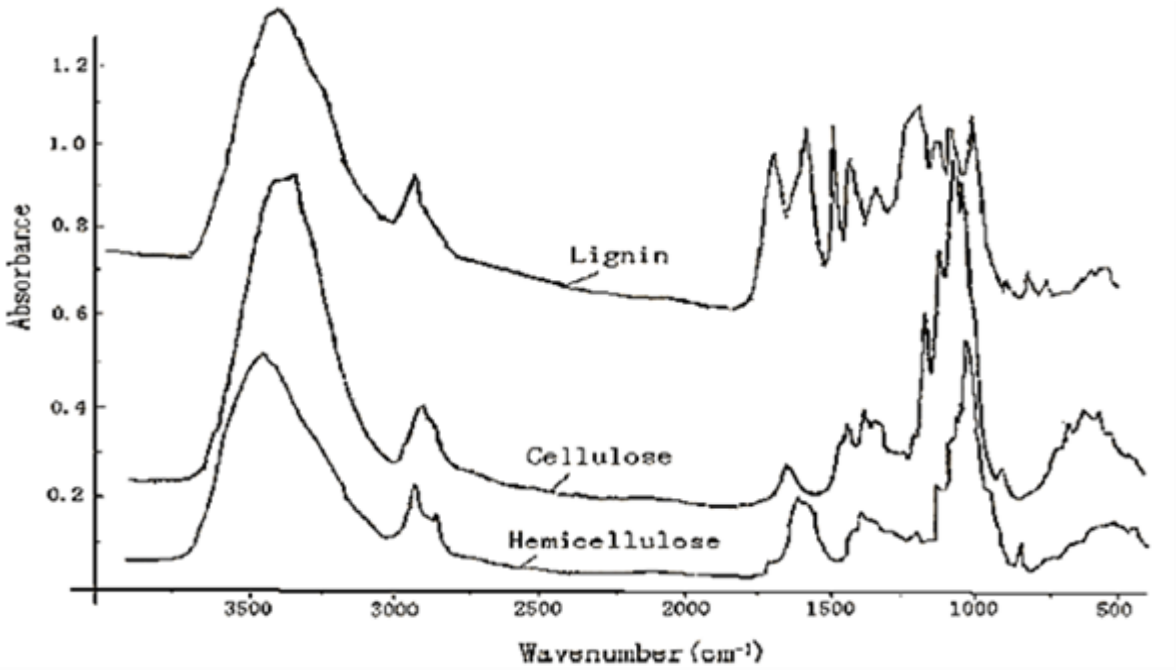


Figure 1.15. IR spectra of cellulose, hemicelluloses and lignin of natural fibres (Liang and Marchessault 1959).

The basic components of an FTIR are shown schematically in **Figure 1.16**. Basically an FTIR Spectrometer collects and digitizes the interferogram, performs the FT function, and displays the spectrum. At first an interferogram of a sample signal is collected using an interferometer. The interferometer consists of a beam splitter, a fixed mirror, and a mirror that translates back and forth; radiation from the IR light source strikes the beam splitter and separates into two beams. One beam is transmitted through the beam splitter to the fixed mirror and the second is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beam splitter. Again, half of this reflected radiation is transmitted and half is reflected at the beam splitter, resulting in one beam passing to the detector and the second back to the source. Then a Fourier Transform (FT) is performed on the interferogram to obtain the spectrum and finally the spectrum is displayed to the screen (Griffiths and de Hasseth 2007).

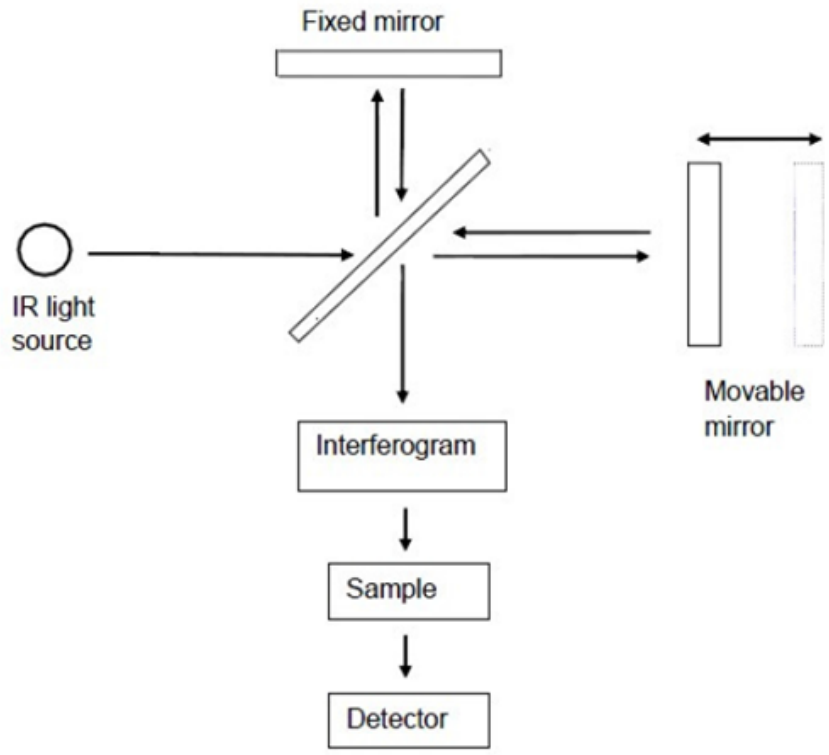


Figure 1.16. Operating principle of FTIR spectrometer (Griffiths & de Hasseth 2007).

FTIR spectra can be obtained directly (with no sample preparation) on solid samples such as wood, pulp, and paper by attenuated total reflectance (ATR), diffuse reflectance (DRIFT), and photoacoustic (PAS) techniques.

Attenuated total reflection (ATR) FTIR uses crystal made typically of Zinc Selenide, Germanium or Diamond. For measuring the solid or the liquid sample is placed on ATR crystal then a beam of infrared light is passed through the ATR crystal and some part of sample (**Figure 1.17**). The wave which extends into the sample is called evanescent wave. The angle of incidence must be greater than the critical angle for total internal reflectance of the infrared light. When the beam exits the crystal, it is collected by a detector and analysed and displayed in form of the ATR-IR spectra.

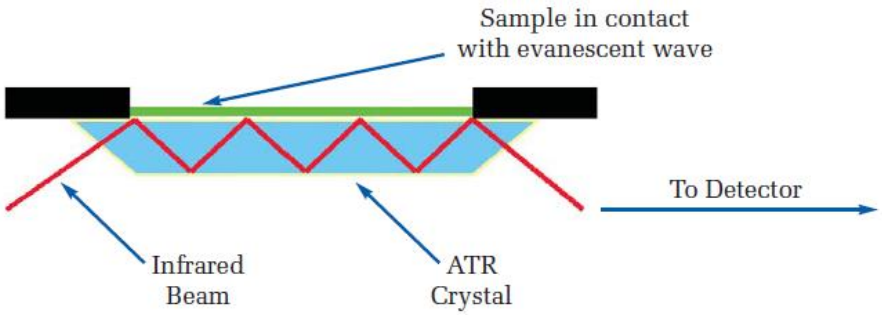


Figure 1.17. A multiple reflection ATR system **(**http://www.utsc.utoronto.ca/~traceslab/ATR_FTIR.pdf).

Infrared radiation is electromagnetic radiation which covers the wavenumbers between 14000 cm^{-1} and 10 cm^{-1}. The infrared region is usually divided into 3 regions: far-infrared (10–400 cm^{-1} or 30–1000 μm) middle-infrared (400–4000 cm^{-1} or 2.5–30 μm) and near-infrared (4000–14000 cm^{-1} or 0.8–2.5 μm) regions. Organic compounds have fundamental vibration bands in the middle-infrared region, which is why the region is widely used in infrared spectroscopy (Griffiths and De Haseth 2007).

An infrared spectrum is unique to each substance and can hence, in principle, be used as an unbiased characteristic to identify the sample. Therefore infrared spectroscopy is one way of knowing or determining the structure of organic compounds. It can also determine the quality or consistency of a sample and the amount of components in a mixture.

Lignin infrared spectra consist of 20 main asymmetric absorption bands, which are typical for high-molecular compound with irregular structure. Spectrums of lignin IR spectra differ from each other in band's intensity, but the number of bands and their frequencies are similar (Sarkanen and Ludwing 1971).

There is a strong wide band between 3500 – 3100 cm^{-1}, typical for every lignin IR spectrum, assigned to OH stretching vibrations which are caused by presence of alcoholic and phenolic hydroxyl groups involved in hydrogen bonds. Absorption bands caused by C─H stretching vibrations of methyl, methylene or methane group are located near 2940 and 2840 cm^{-1} (Durie and Lynch 1960).

Hergert assigned bands in the 1765-1615 cm^{-1} wavenumber range to stretching vibrations of the carbonyl groups. The absorption band located at 1660 cm^{-1}, originated from=O stretch a ketone group located at α-position in unconjugated ketone (β-carbonyl) were observed in all spectra at

1710 cm^{-1}. Stretching vibrations of C═C bonds are found in the 1608-1626 cm^{-1} region. Aromatic vibration in the lignin fraction is present in the bands 1400 to 1600 cm^{-1} (Hergert 1960).

The band at 1460 cm^{-1} indicates the deformation vibrations of CH_2-groups and aromatic ring vibrations. The absorption band at 1420 cm-1 is related to methoxyl groups, and absorption band at 1455 cm^{-1} is related to CH_3 in acetyl groups. The strong intensities of the band at 1329 and 1122 cm^{-1} are associated with syringyl structure in the lignin molecule (Durie and Lynch 1960).

Studies of lignin model compounds made it possible to assign the absorption bands at 1380 and 1340 cm^{-1} to phenolic hydroxyls. Absorbance near 1315 cm-1related to syringyl, which is typical for hardwood lignin and the bands at 1225, 1034 are associated with guaiacyl units in lignin molecules. The intensity increase of the absorption band between 1240-1210 cm^{-1} in the spectra of methylated and acetylated lignin allows relating the band to asymmetric stretching vibrations of the C─O─C linkages in ethers and esters or to phenolic hydroxyls after having studied model compounds (Sakakibara and Sano 2000).

Hergert reports, that the bands at 1190, 1125 and 1031 cm^{-1} originates from methoxyl groups and the bands at 1090-1075 and 1040 cm^{-1} emanates from primary and secondary alcoholic groups. The absorption band located in 988-960 cm^{-1} region relate to deformation vibrations of C─H bonds related to double bonds cm^{-1}. In the region 900-700 cm^{-1} absorption bands caused by deformation vibrations of C-H- bonds on the benzene ring are located (Hergert 1960).

Assignment of main infrared spectral bands data presented in **Table 5** are a collection from selected sources (Sarkanen and Ludwing 1971; Durie and Lynch 1960; Hergert 1960; Sakakibara and Sano 2000)

Table 5. Assignment of Main Infrared Spectral Bands for Lignin

3500 – 3100 cm^{-1}	O-H stretch, H-bonded
2940 , 2840 cm^{-1}	C─H stretch of the methyl, methylene or methane group
1765-1615 cm^{-1}	stretching vibrations of carbonyl groups
1712 cm^{-1}	C=O stretch, unconjugated ketone, carboxyl, and ester groups
1660 cm^{-1}	C=O stretch in conjugated ketone
1608-1626 cm^{-1}	Stretching vibrations of C═C bonds
1600 cm^{-1} to 1400 cm^{-1}	vibrations of aromatic rings present in lignin
1585-1580 cm^{-1}	aromatic rings, conjugated with α-carbonyl group
1460 cm^{-1}	deformation vibrations of CH_2-groups
1455 cm^{-1}	CH_3 in acetyl groups

1420 cm^{-1}	deformation vibrations of the CH-group in the aromatic ring
1380 , 1340 cm^{-1}	phenolic hydroxyls
1365 cm^{-1}	symmetric deformation vibrations of C─H in metoxyl groups
1315, 835-815 cm^{-1}	Syringyl units in hardwood lignin molecules,
1240-1210 cm^{-1}	asymmetric stretching vibrations of the C─O─C linkages in ethers and esters or to phenolic hydroxyls
1225, 1034 cm^{-1}	guaiacyl units in lignin molecules
988-960 cm^{-1}	deformation vibrations of C─H bonds related to double bonds
900-700 cm^{-1}	deformation vibrations of C-H- bonds on the benzene ring are located

2 EXPERIMENTAL SESSION

2.1 Materials

2.1.1 Aspen wood

Dry ground aspen wood was the reference material for aspen wood pulp.

2.1.2 Aspen wood pulp

Commercially available aspen wood pulp from Estonian Cell Company was also the starting material of all the experiments in the present work. BCTMP - Bleached-Chemi-Thermo-Mechanical aspen pulp. Aspen 350/84 B, where 350 is degree of refining and B is brightness level.

2.1.3 Cellulose binder

Cellulose binder is 100% cellulose powder with a particle size of less than 20 μm. Chemical formula $(C_6H_{10}O5)_n$. Used for making quantification standards of lignin.

2.2 Chemicals

2.2.1 Benzene

Benzene was supplied from Sigma Aldrich with 96.0% purity and used for lignin isolation form wood and pulp. Molecular weight 78.11 g/mol. **Figure 2.1** shows structure of benzene.

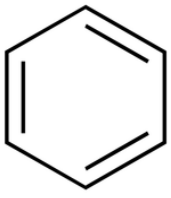


Figure 2.1. The structure of benzene

2.2.2 Sulphuric acid

96.0% Sulphuric acid (H_2SO_4) was supplied from Sigma Aldrich and used for lignin isolation form wood and pulp. Molecular weight is 98.08 g/mol.

2.2.3 Phloroglucinol

A solution of phloroglucinol in strong hydrochloric acid is known as Weisner reagent. Phloroglucinol was supplied from Sigma Aldrich with 99.0% purity and used for staining reactions. Molecular weight 126.11 g/mol. **Figure 2.2** shows the structure of phloroglucinol.

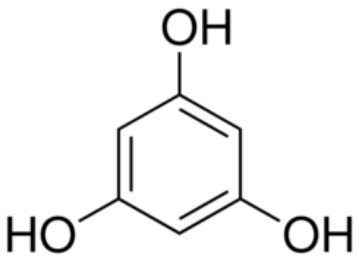


Figure 2.2. The structure of phloroglucinol

2.2.4 Hydrochloric acid

37% Hydrochloric acid, ACS reagent, was supplied from Sigma Aldrich with and used for staining reactions. Molecular weight is 36.46 g/mol.

2.2.5 Ammonium hydroxide

Ammonium hydroxide solution ACS reagent, 28.0-30.0% NH_3 basis was provided by the Chair of Inorganic Materials.

2.2.6 Other chemicals

Potassium permanganate and ethanol were all provided by laboratory. Distilled water was readily used from the laboratory's own distilled water system.

2.3 Apparatus

JASKO UV/VIV/NIR spectrophotometer V-670 (Department of Materials Science) and FTIR Alpha spectrometer using platinum ATR (Laboratory of Inorganic Materials) were used**.**

2.4 Preparation of samples

2.4.1 Aspen wood

The preparation of extractive-free wood for lignin isolation procedure was performed according to TAPPI Standard T264. Method involves three extraction steps; at first with of ethanol-benzene solvent, then with 95% ethanol and finally with distilled water. Before the extraction, fresh sawdust was made of aspen wood with handsaw in the laboratory is, shown in **Figure 2.3**.

Figure 2.3. Raw material: Aspen wood and aspen sawdust

Afterwards, fresh sawdust of about 30g (80 meshes) was extracted with 400 ml of ethanol-benzene solvent (1:2 by volume) in a flask reactor with a reflux condenser (**Figure 2.4**) for 6 hours, keeping the liquid stably boiling. Benzene is highly flammable liquid and toxic, therefore should handle with care and under ventilation.

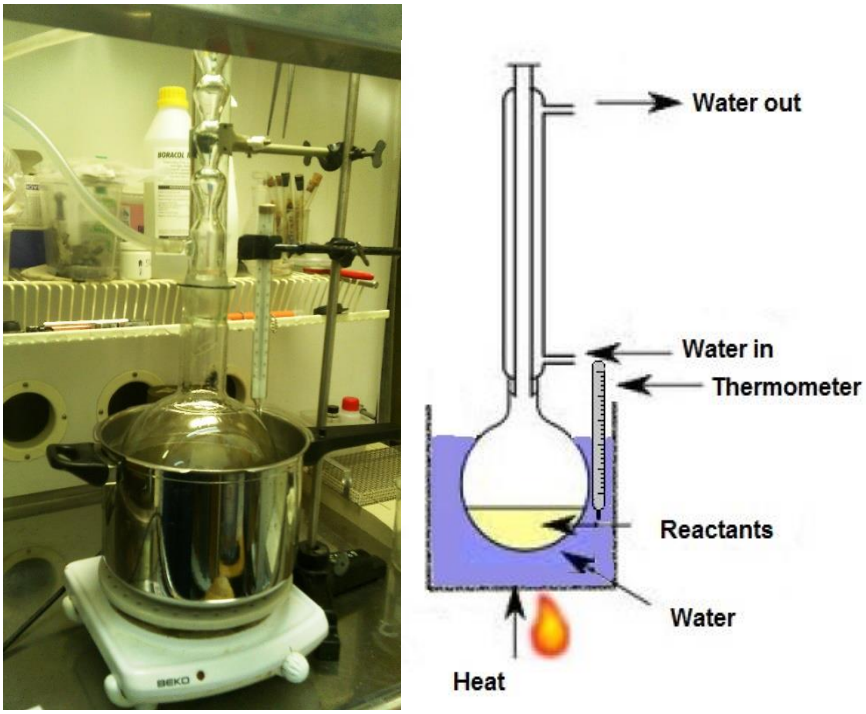


Figure 2.4. Flask reactor with Reflux condenser

After extraction with ethanol-benzene, the excess solvent was removed with suction on a Büchner funnel and then the sample was washed with ethanol to remove the benzene. After that, sawdust was returned to the extraction flask and extracted with 95% ethanol for 4 hours.
Then the sample was filtered and washed with distilled water to remove the ethanol.

Finally, the sample was transferred to a 1000-ml Erlenmeyer flask and 500 ml of boiling distilled water was added. The flask was heated for 1 hour in the water bath at boiling temperature.

After extraction, the sawdust sample was filtered on a Büchner funnel, while washed with 500 ml of boiling distilled water. Then the sample was allowed to air-dry thoroughly at room temperature (TAPPI T264).

2.4.2 BCTMP

All BCTMP samples were oven dried at 105 ºC and grinded in a coffee-grinder. **Figure 2.5** shows dried BCTMP of aspen from paper mill and dried and grinded pulp.

Figure 2.5. Photo of dried pulp mass from paper mill (left) and dried and grinded pulp (right).

2.5 Lignin determination

2.5.1 Determination of Acid Insoluble (Klason) Lignin

Before determination of lignin in samples started, acid-insoluble lignin was first isolated from extractive-free aspen wood and also from BCTMP pulp. The isolation procedure was performed based on TAPPI Standard T222. Acid-insoluble was weighted by analytical scale and analysed with FTIR spectrometer.

2.5.1.1 Isolation acid-insoluble lignin from aspen wood and BCTMP

For isolating lignin from the extractive-free sawdust sample with 1 g for wood and 2 g for pulp dry weight was prepared. The sample was placed into 100-ml beaker, 15 ml (for wood) 40 ml (for pulp) of cold (10 to 15°C) 72% sulphuric acid was added. Sulphuric acid was added gradually in small increments while the material was stirred and macerated with a glass rod. Beaker was kept in a bath at 20±1°C during dispersion of the material. After the sample was dispersed, beaker was covered with a watch glass and was kept in a bath at 20±1°C for 2 hours. The material was stirred frequently during this time to ensure complete dissolution.

Afterwards, the solid solution was transferred from the beaker to the flask and about 300 ml of water was added to the flask. Then more water was added to dilute the solution to a 3% concentration of sulphuric acid, to a total volume of 575 ml for wood and 1540 ml for pulps. Then the solution was boiled for 4 hours by using a flask reactor with a reflux condenser. After that, the lignin was transferred to the filter and hot water was used to wash, and lignin was dried at room temperature (TAPPI T222).

For each determination, the lignin content in the test specimen was calculated as follows:

$$\boldsymbol{Lignin}, \% = \frac{\boldsymbol{A \times 100}}{\boldsymbol{W}} \qquad [5]$$

Where:

A = weight of lignin, g

W = oven-dry weight of test specimen, g

2.5.2 FTIR spectroscopic experiments

FT-IR spectra were recorded on Alpha spectrometer using platinum ATR (**Figure 2.6**) in the Laboratory of Inorganic Materials. The study was carried out over the wavelength range 400 to 4000 cm^{-1}. All solid samples must be dried to stable weight before analyse to avoid absorbance caused by water molecules. The sample can be measured directly with ATR (attenuated total reflection) technique, no further sample preparation was needed.

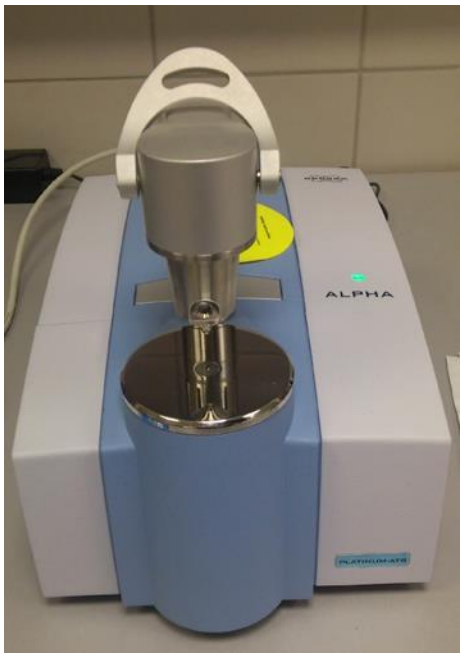


Figure 2.6. Photo of Alpha FTIR spectrometer using platinum ATR

The spectra of starting materials of this work: aspen wood and BCTMP were recorded, analysed and compared. For calibration of lignin for staining reactions, microcrystalline cellulose binder and aspen extractive-free powder were used. Cellulose with different amount of aspen (0%, 25%, 50%, 75%, 100% wt. % of aspen) were mixed together and analysed with FTIR. To compare with colour reactions an average content 21 % of lignin was assigned to experimental content of aspen wood powder/microcrystalline cellulose binder mixtures.

2.5.3 UV spectroscopic experiments

UV-VIS spectroscopic experiments for solid samples were performed in the Department of Materials Science of TUT. The diffuse reflectance spectra from samples pressed into pellets were recorded at room temperature on a JASKO UV/VIV/NIR spectrophotometer V-670 equipped with an integrating sphere (**Figure 2.7**). The reflectance spectra were recorded against $BaSO_4$ as a white (R_∞) optical standard. The study was carried out over the wavelength range 250 to 600 nm. The reflectance spectra of woody samples were converted into pseudo-absorption k/s spectra using the Kubelka-Munk equation 4.

Figure 2.7. Photo of JASKO UV/VIV/NIR spectrophotometer V-670 (http://www.ttu.ee/keemia-ja-materjalitehnoloogia-teaduskond/materjaliteaduse-instituut/teadusaparatuur/)

For the quantification of lignin content for staining reaction a series of extractive-free aspen wood powder/microcrystalline cellulose binder were mixed (0%, 25%, 50%, 75%, 100% wt. % of aspen) and pressed into pellets (**Figure 2.8**) for UV experiments. To compare with colouring reaction lignin content in aspen wood powder/microcrystalline cellulose blinder mixtures were assigned (0%, 5.25%, 10.5%, 15.75%, and 21%).

Figure 2.8. Extractive-free aspen wood powder/microcrystalline cellulose binder were mixed (0%, 25%, 50%, 75%, 100% wt. % of aspen) and pressed into pellets for UV experiments

The choice to use aspen wood powder/microcrystalline cellulose binder mixtures for the quantification of lignin content both for FTIR-ATR and UV-VIS experiments, was due to the failure in colouring experiments of experimentally obtained aspen wood Kraft lignin and purchased (Aldrich) Kraft lignin. Even though this method is not exact, it allows fast overview about delignification rate along chemical modification of woody material. According to the literature (Gray 1971) modified lignin may fail in colouring reactions due to the strongly modified structure. Also measurement of lignin content in liquid extracts does not correspond to the remained lignin in solid residue.

2.5.1 Staining experiments

The main aim for staining experiments was estimating the presence of lignin in aspen wood and BCTMP. Weisner and Mäule Reaction were chosen for colouring the samples. According to literature Weisner Reaction is fast procedure of good specificity and that can be used on wood and pulp, lignified tissues establish violet colour. Using Mäule test hardwood and softwood lignin can be differentiated with this reaction that give a distinctive red colour if derived from a hardwood species and to brown colour to softwood lignin. Based on literature these methods were estimated suitable for staining experiments to detect lignin in aspen wood and BCTMP (Gray 1971).

Since lignin has a complex structure and vary among the wood species, also different treatments can affect colouring reactions and literature did not provide exact parameters for dry aspen wood and BCTMP staining, which give the best colour intensity in suspension, therefore best colouring conditions for our samples were determined. Reaction time and ratio between mass of sample and volume of solvent used for staining which give the best colour intensity were determined. For experiments samples (with mass of 0.025g to 0.1 g) were weighted to glass test-tubes. Suspension containing weighted samples and staining solutions with total volume of 1ml to 2 ml were made. All suspensions were photographed to be analysed visually.

For Weisner Reaction first a 2% (w/v) solution of phloroglucinol in 95% ethanol was prepared and stored in a sealed bottle in the dark. Then phloroglucinol solution with 20% hydrochloric acid was mixed or added separately and applied to test samples in glass test-tubes. The red colour ($\lambda max \sim 550$ nm) should become apparent immediately (2-3 minutes), but starts to fade with time. Since in literature (Gray 1971) suggest that phloroglucinol and HCl should be mixed before added to samples, difference between mixed and separately added solution to samples was examined. To investigate how our sample mass is dependent on solution volume used for staining, three samples (both aspen wood and BCTMP) with different mass (0,1 g, 0,05 g and 0,025g) in 1 ml staining solution were examined. Reference sample were treated only with phloroglucinol solution and without reagent hydrochloric acid. To determine the best ratio between phloroglucinol/HCl for aspen wood and BCTMP samples, experiment with three ratios 2:1, 1:1 and 1:2 was carried out, photos were taken after 20 seconds; 2, 5 and 15 minutes to show effect on reaction time with these different ratios.

For Mäule Reaction samples were immersed in 1% (w/v) aqueous potassium permanganate for 5 to 10 minutes. Then filtered using filter-paper and washed with 5 ml distilled water, and treated with 3% aqueous hydrochloric acid until the colour changes from black to beige. When the dark colour has been fully discharged the samples were filtered and washed again with 5 ml distilled water. Finally concentrated ammonium hydroxide was added to develop a magenta colour in hardwood samples. Colour intensity between aspen wood and BCTMP samples with 1ml and 2ml NH_4OH solution were investigated.

To make visual standard suspension series, mixtures of extractive-free aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) were stained using Weisner and Mäule reaction.

3 RESULTS AND DISCUSSION

3.1 Analysis of materials

3.1.1 Aspen wood and BCTMP

FTIR spectra of aspen wood and BCTMP were recorded and compared (**Figure 3.1**). For better readability, mean spectra were offset along the absorbance axis and presented on the same scale. The horizontal grey line indicates the zero value for aspen wood spectrum.

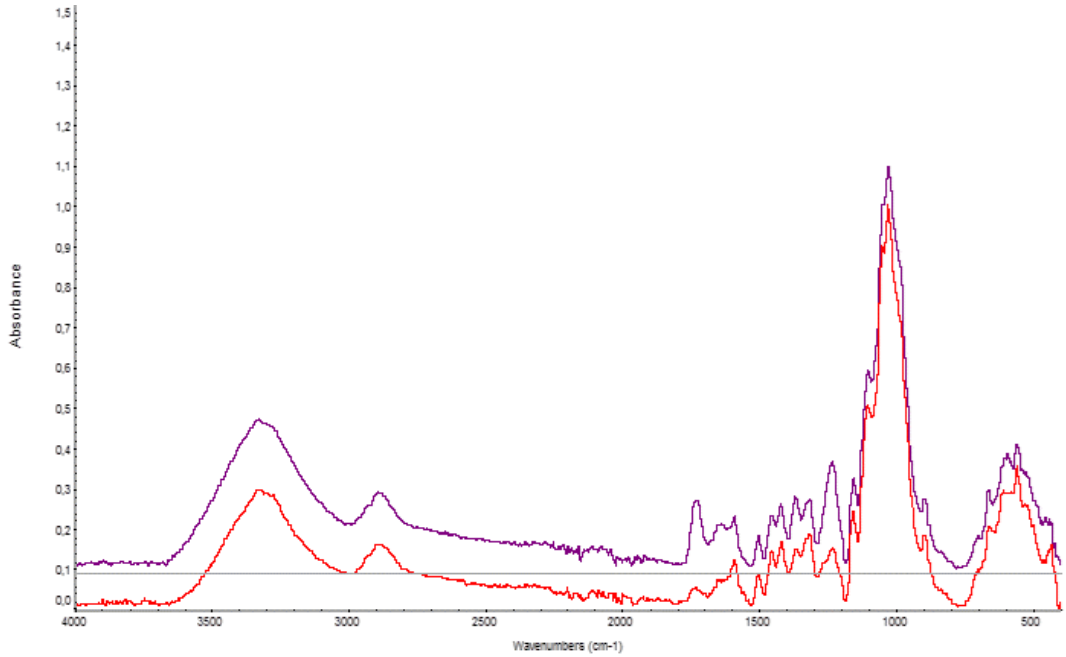


Figure 3.1. The IR spectra of aspen wood (violet) and BCTMP (red).

The spectrum of aspen wood and BCTMP were quite similar and showed main peaks which are to wood spectra - strong broad OH stretching (3500 – 3100 cm^{-1}), C–H stretching in methyl and methylene groups (2800–3000 cm^{-1}), and a strong broad superposition with sharp and discrete absorptions in the region 1000 cm^{-1}(Durie and Lynch 1960).

Comparison of the aspen wood and BCTMP spectrums in aspect of lignin reveals main differences between absorptions situated 1735 -1740 cm^{-1} wavenumber range which shows there are more aliphatic aldehyde and ester group present in aspen wood than BCTMP. Also absorption intensity in aspen wood spectrum is stronger than in BCTMP spectrum 1234-1237 cm^{-1} wavenumber range which according to literature is due asymmetric stretching vibrations of the C─O─C linkages in ethers and esters or to phenolic hydroxyls which are associated with lignin.

Preconditioning Refiner Chemical - Alkaline Peroxide Mechanical Pulp process for making BCTMP is lignin non-destructive process and with comparison with aspen wood, removed lignin can be detected using FTIR, but exact quantification is difficult.

3.2 Klason lignin isolated from aspen wood and BCTMP

To determine acid-insoluble lignin content of the extractive-free aspen sawdust and BCTMP, the procedure described in TAPPI T222 standard was followed. My experiment's Klason lignin extractions from the aspen wood and BCTMP are shown in the **Figure 3.2.**

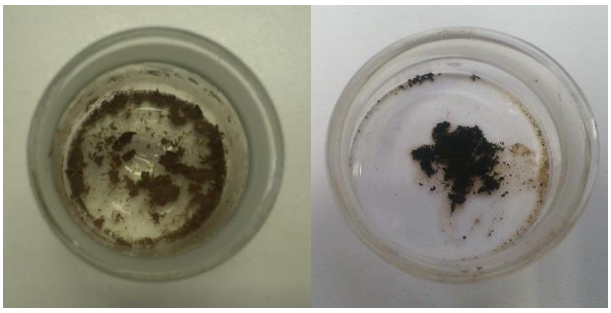


Figure 3.2. Photo of aspen wood lignin (left) and BCTMP lignin (right).

The shape and colour of Klason lignin had obvious differences between wood and BCTMP. The colour of Klason lignin in aspen wood was brown and had bigger particles whereas the colour in BCTMP was black and the particles were smaller, which was probably result of mechanical treatment and bleaching chemicals used for making BCTMP from aspen wood.

Moreover, the Klason lignin was isolated from about 1 g of dry weight of sawdust and 2 g of BCTMP, then the acid-insoluble Klason lignin contents were calculated in the samples by using the equation 5 (TAPPI T222 om-06).

Aspen wood lignin, % =15,
BCTMP lignin, % = 10,

According to the literature (Dean 1997), there is approximately 21% of lignin aspen wood and aspen bleached chemi-thermomechanical pulp contains 17% lignin. Some of lignin isolated from BCTMP could not be filtered out using this method. Also as said in the literature the lignin remaining in solution after the sulphuric acid has been diluted and may represent as much as 3-5% of the total lignin (Dean 1997; Reis and Nielsen 2001).

3.3 Staining reactions

3.3.1 Calibration of lignin for staining reactions

To compare with colour reactions calibration with FTIR and UV-VIS- spectrometer were made, an average content 21 % of lignin was assigned to experimental content of 100% aspen wood in aspen wood powder/microcrystalline cellulose binder mixtures.

FTIR absorbance of measured samples showed maxima at 1234-1237 cm^{-1} this was taken as measurement point for the calibration of lignin content. For better readability, mean spectra were offset along the absorbance axis and presented on the same scale. The horizontal grey lines indicate the zero value for each spectrum (**Figure 3.3**). Near linear dependence was established (**Figure 3.4**)

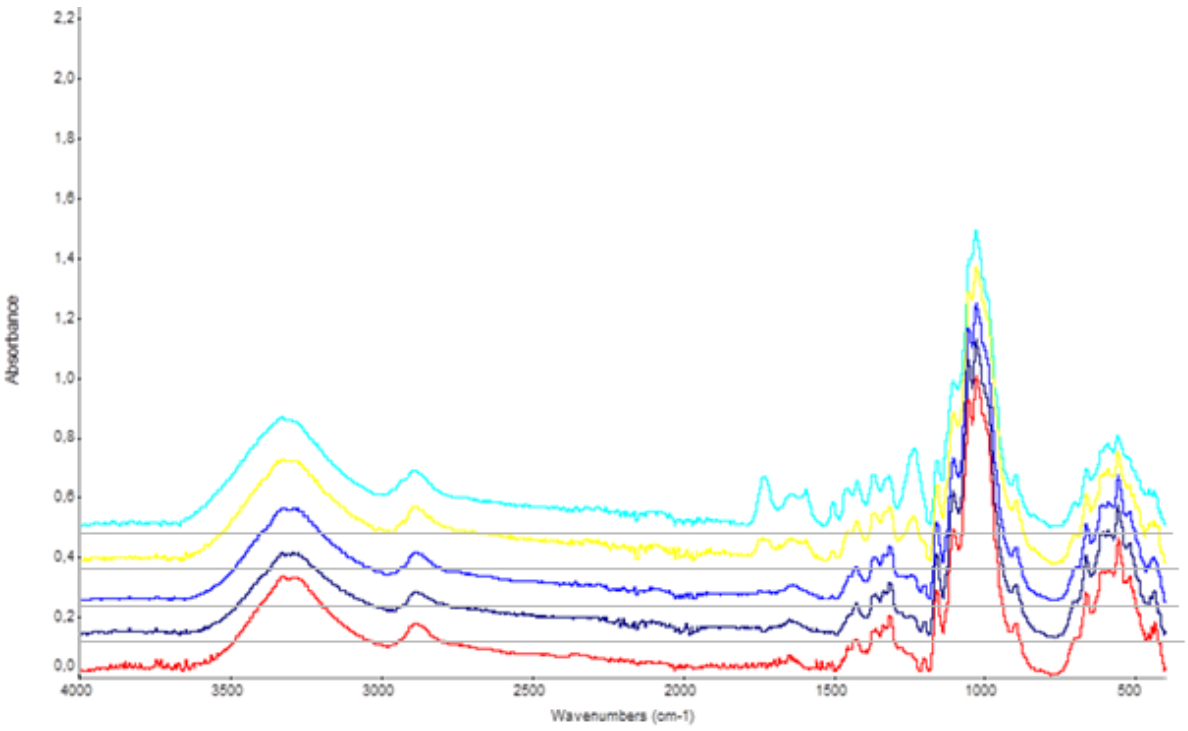


Figure 3.3.FTIR spectra of : 100% cellulose binder (red),25% wood + 75% cellulose binder (dark blue), 50% wood + 50% cellulose binder (blue), 75% wood + 25% cellulose binder (yellow), 100% wood (cyan).

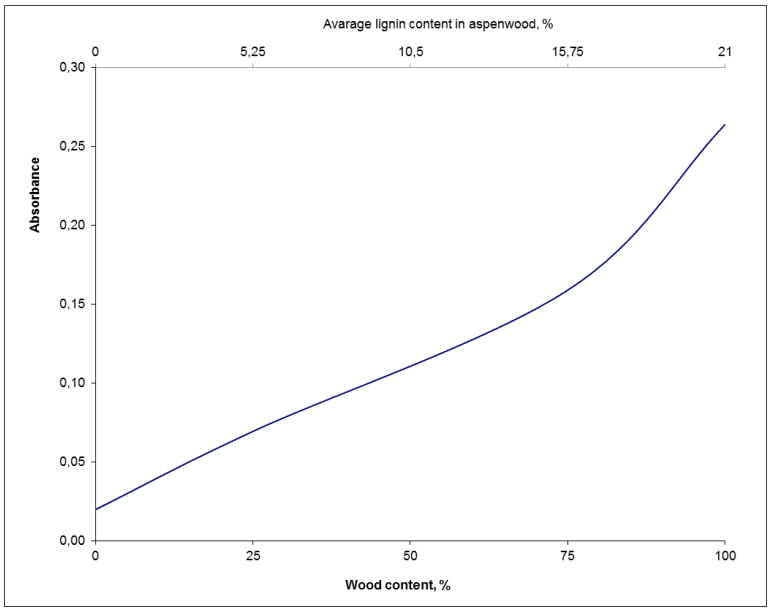


Figure 3.4. Calibration curve for the detection of lignin content at maxima between 1234-1237 cm^{-1} for the comparative experiments of lignin colouring.

UV-VIS pseudo-absorbance of measured samples showed distinct maximum at 280 nm and this was taken as measurement point for the calibration of lignin content (**Figure 3.5.**). Near linear dependence was established (**Figure 3.6**).

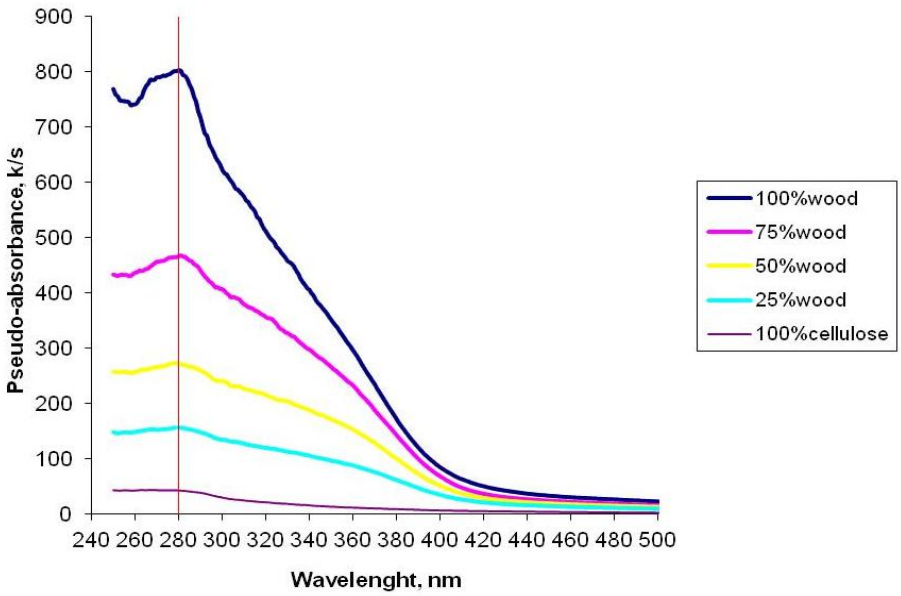


Figure 3.5. Pseudo-absorbance spectra of aspen wood powder/microcrystalline cellulose binder mixtures. Distinct absorbance maximum at 280nm was detected.

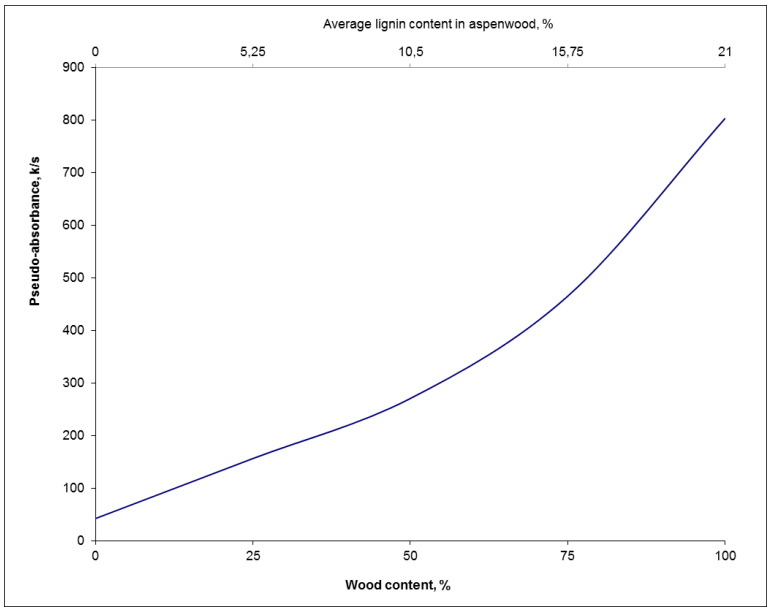


Figure 3.6. Calibration curve for the detection of lignin content at 280 nm for the comparative experiments of lignin colouring.

3.3.2 Weisner reaction

At first, the difference between mixed and separately added phloroglucinol and reagent HCl (with 2:1 ratio) to 0.05 g of aspen wood (a) and BCTMP (b) samples was investigated. Photo was taken after 3 minute staining reaction. As can be seen in **Figure 3.7** there is practically no visual difference between phloroglucinol and HCl are mixed together (on the left in photos a and b) or not (on the right) before added to samples. Therefore staining solution can be applied to samples separately which makes the procedure quicker.

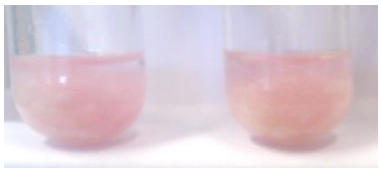
a.  b. 

Figure 3.7. Photos show no colour difference between suspension samples of aspen wood(a) and BCTMP(b) stained with mixed phloroglucinol/HCl reagent before(left) applied to sample or added separately (right), reaction time 3 minutes.

Next aim was to find best reaction time and ratio between phloroglucinol/HCl. Literature suggested (Dean 1997) to add 2 parts of phloroglucinol and one part of HCl to samples. Gray suggested to add more reagent if colour starts to fade (Gray 1971). Our previous experiments showed that adding more reagent HCl to samples will increase the intensity of the colour in suspension, therefore three different phloroglucinol/HCl ratios: 2:1, 1:1 and 1:2 were investigated. As can be seen in figure 3.7 both aspen wood (c) and BCTMP (d) samples

established the quickest (c.1 and d.1) intensive colour with phloroglucinol/HCl ratio 1:1 (in the middle of photo c.1 and d.1), ratio 2:1 that was suggested in literature (on the left) was the slowest and did not establish same intensity as 1:1 even in 5 minutes (c.3 and d.3). Colour intensity for ratio 1:1 and 1:2 was same after 2 minute reaction for aspen wood and after 5 minutes for BCTMP samples staring from. For BCTMP samples there were big difference between reaction time 2 minutes (c. 2) and 5 minutes (c.3). In 20 minutes colour starts to move from fibre into solution as can be seen in photos c.4 and d.4. Therefore it is advisable to add more reagent if suspensions are investigated.

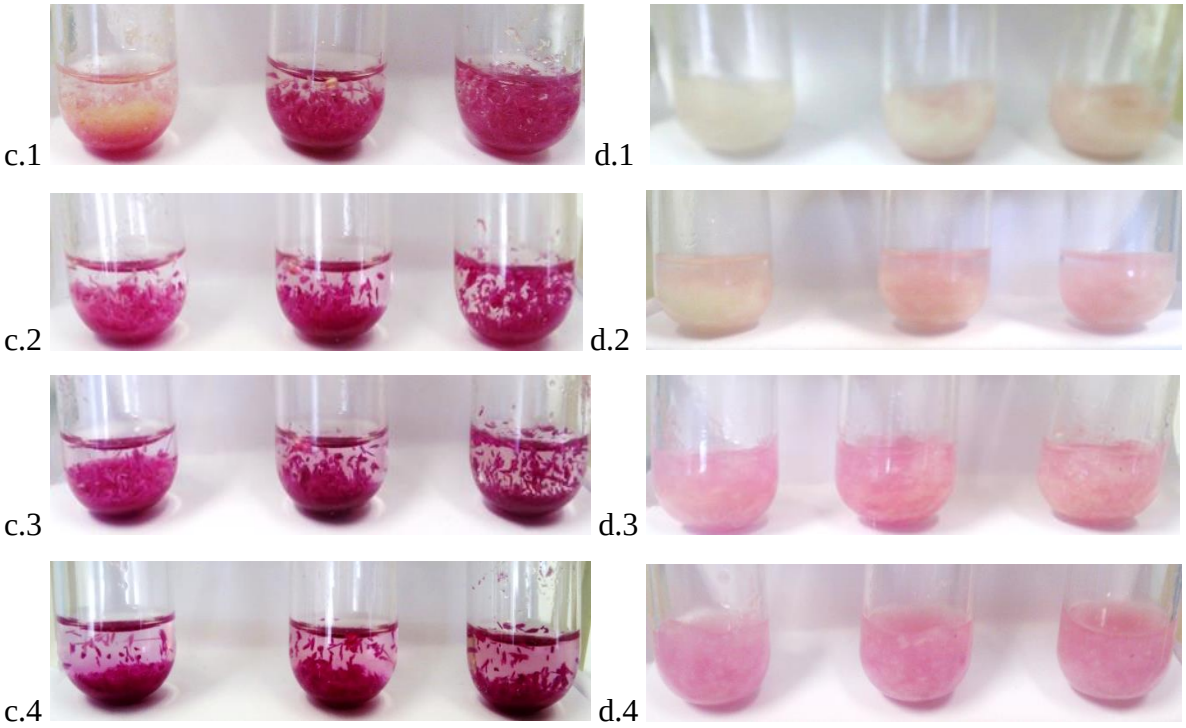
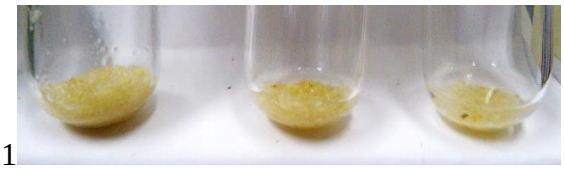
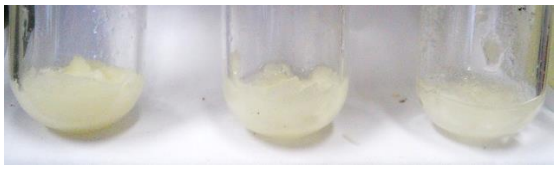


Figure 3.8. Photos show the effect of different phloroglucinol/HCl ratio 2:1, 1:1, and 1:1 on suspension samples of aspen wood (c) and BCTMP (d) stained with phloroglucinol/HCl reagent, starting from top samples stained for 20 s, 2 min, 5 min and 20min.

Dependence on ratio between mass of sample and staining solution volume was also investigated (**Figure 3.9**). Photos e.1 and f.1 show samples with only phloroglucinol and without reagent. As can be seen in photos e.2 and f.2 increasing the mass of sample will make colour more intensive and best result both for aspen wood (e.2 left) and BCTMP (f.2 left) were with established 0.1g of sample in 1 ml staining solution. Therefore, it can be said that mass of sample is dependent on solution volume and to get comparable results in suspension samples must be with same weighted before staining reaction. Also it was noticed during this experiment, that carefully mixing the samples during staining reaction will allow colour in suspension to be evenly distributed.

e.1  f.1 

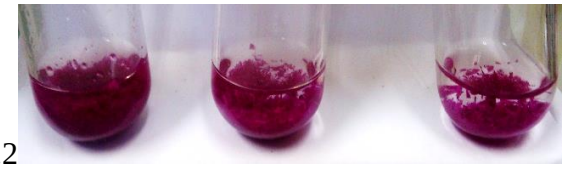
e.2  f.2 

Figure 3.9. Photos show different amount of aspen wood (e) and BCTMP(f) (from left 0.1g, 0.05g, 0.025g) suspension samples stained with phloroglucinol/HCl reagent, top without reagent HCl(e.1;f.1), bottom stained(e.2;f.2).

Visual suspension series for Weisner reaction were made with mixtures of extractive-free aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) can be seen on **Figure 3.10**. Since aspen wood powder and microcrystalline cellulose binder had different particle size, calibration series were not as clearly distinguishable as wanted.

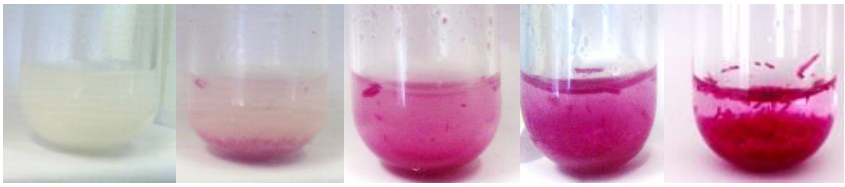


Figure 3.10. Photos show suspension of aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) samples stained with phloroglucinol/HCl .

3.3.3 Mäule reaction

Compared to Weisner reaction, Mäule colouring test is more complicated and involves staining with three different solutions: $KMnO_4$, HCl, and finally NH_4OH. After treatment with first and second solution, samples should be washed with distilled water.

In first step of Mäule reaction samples of wood (left in the photo g and h) and BCTMP (right) turned brown/black in 10 minutes (h) after 1% KMnO4 solution was added (**Figure 3.11.**).

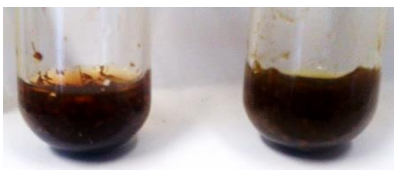
g  h 

Figure 3.11. Photos show suspension of aspen wood (left) and BCTMP (right) samples treated with 1% KMnO4 solution for 2 minutes (g) and 10 minutes (h)

After washing with distilled water, 3% HCl was applied to samples. Aspen wood (left) and BCTMP (right) brown colour turned to beige/yellow (j) after 2 minutes. Then samples were washed again.

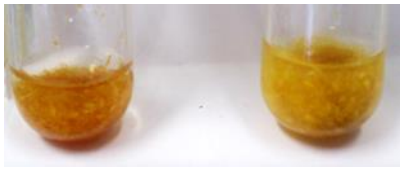
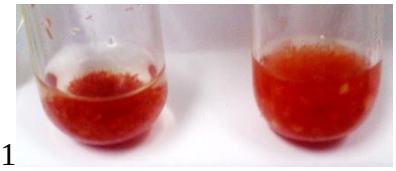
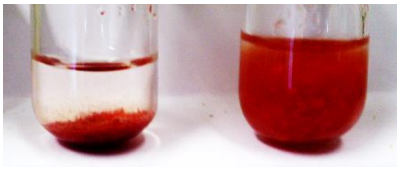
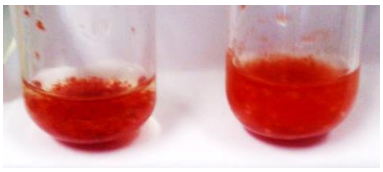
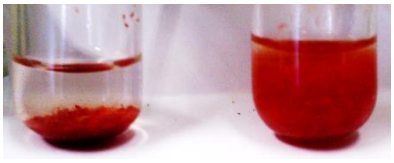
i  j 

Figure 3.12 Photos show suspension of aspen wood (left) and BCTMP (right) samples treated with 3 % HCl solution for 20 s (a) and 2 minutes (b).

As can be seen on **Figure 3.13** intensive red colour comes both with aspen wood(left) and BCTMP (right) samples in 1ml and 2 ml solution in 1 minute (k.1 and l.1) and starts to diffuses out of the tissue into solution in 10 minutes (k.3 and l.3). There is only slight difference between aspen wood (left) and BCTMP (right) samples colour intensity, bigger difference in colour intensity is between staining solution volume 1 ml (a) and 2 ml (b). Samples stained with 2 ml NH_4OH have more intensive colour in suspension than samples coloured with 1ml solution.

k.1  l.1 

k.2  l.2 

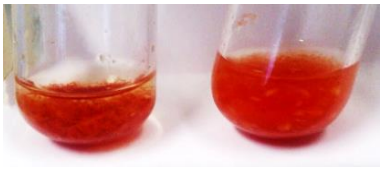
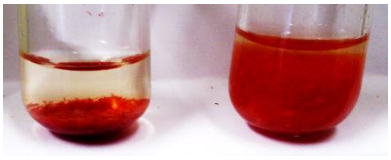
k.3  l.3 

Figure 3.13. Photos show Mäule reaction´s third step - suspension containing samples with 1ml (a) and 2ml (b) NH_4OH solution. Reaction time: 1 minutes (k.1; l1), 5 minutes (k.2; l.2) and 10 minutes (k.3; l.3).

Visual suspension series for Mäule reaction was made also with mixtures of extractive-free aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen). Since Mäule reaction involves steps with three solutions, 1% KMnO4 solution (m) 3 % HCl solution (n) NH_4OH solution (o) with different colour reaction to samples, all of these were photographed (**Figure 3.15.**). Because cellulose binder was with a particle size of less than 20 μm, for washing and filtering qualitative filter-paper was used, photos of 1% $KMnO_4$ filtering process are shown in **Figure 3.14**.

Figure 3.14. Photos of 1% KMnO4 filtering process

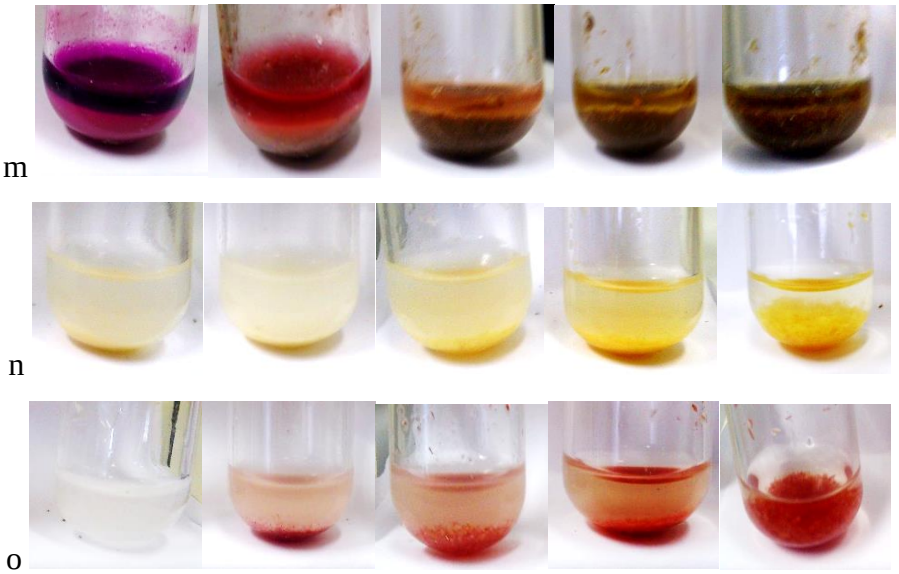


Figure 3.15. Photos show suspension of aspen wood powder/microcrystalline cellulose binder (0%, 25%, 50%, 75%, 100% wt. % of aspen) samples treated with 1% KMnO4 solution (m) 3 % HCl solution (n) NH_4OH solution (o)

CONCLUSIONS

Lignin is organic substance binding the cells with complex structure with variations among the wood species and with differently treated pulps, therefore there is no generally applicable method for determination of lignin. The main objective of this thesis was find effective, simple but quantitative method for estimating the presence of lignin in aspen wood and BCTMP. For that, different methods for determination of aspen lignin were investigated.

- Dried samples of extractive-free aspen wood and grinded BCTMP pulp of aspen were used in experiments.
- For isolating lignin from aspen wood and BCTMP Klason method was used. Using this method was determined that aspen wood sample contained 15 % lignin and BCTMP 10 % lignin. According to the literature, there is approximately 21% of lignin aspen wood and aspen bleached chemi-thermomechanical pulp contains 17% lignin. Some of lignin isolated from BCTMP could not be filtered out using this method. Also as said in the literature the lignin remaining in solution after the sulphuric acid has been diluted may represent as much as 3-5% of the total lignin.
- Spectroscopic methods were used for quantification of lignin content for colouring reaction also for comparison of starting materials. The different band values in IR spectrum of samples were explained based on the literature study. Main difference between aspen wood and BCTMP samples spectrum were absorptions situated 1735 - 1740 cm^{-1} and 1234-1237 cm^{-1} wavenumber range.
- For the quantification of lignin content a series of aspen wood powder/microcrystalline cellulose binder were mixed and analysed with FTIR-ATR for UV-VIS experiment samples were pressed into pellets. To compare with colour reactions an average content 21 % of lignin was assigned to experimental content in 100% aspen wood powder. FTIR-ATR absorbance maximum between 1234-1237 cm^{-1} and UV-VIS pseudo-absorbance of measured samples maximum at 280 nm, these maxima were taken as measurement points for the calibration of lignin content. Near linear dependence was established with both methods.
- Main focus was on staining reaction and finding the best methods for identification of lignin in our samples. Weisner and Mäule tests were used for staining reaction to detect lignin in samples. Suspension containing samples with staining solution were made and photographed and analysed. Reaction time and ratio between mass of sample and volume

of solvent used for staining which give the best colour intensity were determined. For Weisner reaction the difference between ratio between phloroglucinol/HCl and mixed and separately added phloroglucinol and reagent HCl was investigated.

- Best positive Weisner reaction with violet color both with aspen wood and BCTMP samples, were established with 1:1 phloroglucinol/HCl staining solution applied to sample and carefully mixed suspension, after 5 min reaction. By increasing mass of sample will increase the colour intensity, also aspen wood samples established more intensive colour than BCTMP, therefore even 5% aspen lignin content differences can be distinguished using this method.
- With Mäule reaction intensive red color to samples game after 10 min treatment with 1% $KMnO_4$ then after washing with distilled water and treated with 3% aqueous HCl until the colour changes from black to beige, washed again and treated with concentrated NH_4OH for 2 minutes. With Mäule reaction both aspen wood and BCTMP samples established intensive red colour, therefore with this method only lignin presence was detectable, but small differences between lignin content in aspen were not distinguished.
- Novelty of this work was quantification of lignin for staining reaction. Even though colouring reaction for lignin identification has been used for an extensive period of time, it has not been calibrated to this day. In this thesis quantification of lignin was carried out by using FTIR and UV-VIS spectrometer. Until now, colouring reactions have been mainly used for detection of lignin in wood. However, in this work Weisner and Mäule reactions proved to be useful for lignin determination in BCTMP.
- Future development involves determination of lignin in smaller quantities by using Mäule reaction. Even though Mäule reaction did not give good results in calibration of lignin, it was more sensitive to hardwood residual lignin than Weisner reaction. In addition, in the future it is possible to use dispersive UV-Raman spectrometer for residual lignin and FTIR-RAMAN for cellulose in instrumental analyses.

The objectives of this thesis were observed. Simple method for detection the presence of lignin in BCTMP was worked out. With Weisner and Mäule test lignin is easily detectable in aspen.

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LIGNIINI MÄÄRAMISE MEETODID HAAVA PUITMASSIS

Kristi Kärner

Referaat

Käesoleva töö eesmärgiks oli leida efektiivne ja lihtne meetod ligniini kvantitatiivseks tuvastamiseks haava puitmassis. Selleks katsetati laboratoorse analüüsi käigus erinevaid ligniini määramise meetodeid. Töös on esitatud teemakohane kirjanduse ülevaade, kirjeldades erinevaid meetodeid ning tutvustab mõningaid selleteemalisi varem läbi viidud uurimusi.

Katseteks valmistati ette kuivatatud ekstratiivainete-vaba haavapuidu ja jahvatatud haava puitmassi (BCTMP) proovid. Ligniini eraldamiseks proovidest kasutati Klasoni meetodit. Spektroskoopilisi meetodeid (FT-IR ja UV-VIS) kasutati ligniini sisalduse määramiseks värvireaktsioonide kalibreerimiseks ning haavapuidu ja BCTMP struktuuri võrdluseks ligniini seisukohast. Töös keskenduti peamiselt värvireaktsioonidele ning nende abil haavapuitmassis ligniini määramiseks parimate parameetrite väljaselgitamisele.

Kirjanduse kohaselt on haavapuidus keskmiselt 21 % ja BCTMP 17 % ligniini, Klasoni meetodiga suudeti määrati ligniini koguseks haava puidus 15% ja puitmassis 10 % . See oli kooskõlas kirjanduse andmetega, kus oli öeldud, et osa ligniini (3-5 % kogu ligniinist) lahustub H_2SO_4 lahjendatud lahuses (nn. happes lahustuv ligniin). Lisaks polnud antud filtreerimismeetodiga võimalik kogu sademes olevat ligniini välja filtreerida.

FTIR-ATR spektromeetriga uuriti haavapuitu ja BCTMP-d. Spektrite analüüs toimus toetudes kirjanduse ülevaates toodud ligniini neeldumismaksimumide paiknemisele. Suurim erinevus haavapuidu ja BCTMP proovide neeldumisspektrites paiknes 1735 -1740 cm^{-1} ja 1234-1237 cm^{-1} vahemikes, kus haavapuidu neeldumismaksimumid olid intensiivsemad BCTMP omadest. Antud maksimumid on seostatavad ligniiniga, järelikult on FTIR-i abil võimalik kaudselt võrrelda ligniini koguse erinevust haavapuidus ja puitmassis.

Värvireaktsioonide jaoks ligniini koguse kalibreerimiseks kasutati haavapuidu pulbri/mikrokristallilise tselluloosi (Cellulose Binder – Ingl.k.) segusid vastavalt haavapuidu mahuprotsendi sisaldusega 0%, 25%, 50%, 75%, 100% ning neeldumisspektrid määrati FTIR-ATR ja UV-VIS spektromeetritega. FTIR- ATR-iga oli võimalik mõõta neeldumisspekter otse proovilt, UV-VISi spektromeetri jaoks pressiti proovidest tabletid ning proovide peegeldumisspektrid muudeti pseudo- neeldumise k/s spektriteks. Värvireaktsioonidega võrdlemiseks valiti keskmiseks haavapuidu ligniini sisalduseks kirjanduse põhjal 21 %. Mõõdetud proovide FTIR-ATR neeldumismaksimum vahemikus 1234-1237 cm^{-1} ja UV-VIS pseudo-neeldumise maksimum 280 nm valiti mõõtepunktiks ligniini koguse kalibreerimiseks. Mõlema meetodiga saavutati peaaegu lineaarne sõltuvus.

Värvireaktsioonidest valiti kirjanduse põhjal välja Weisner ja Mäule testid, mida on kasutatud (puidu)proovides ligniini avastamiseks juba möödunud sajandi algusest alates. Katsete läbiviimiseks valmistati proovi ning värvimislahust sisaldavad suspensioonid, fotografeeriti ja seejärel analüüsiti. Klassikaliste meetodite edasiarendamiseks ja täiendamiseks uuriti värvireaktsiooni intensiivsuse sõltuvust proovi massi ja värvilahuse mahu suhtest, reaktsiooni ajast ja Weisneri reaktsiooni puhul värviaine ja reagendi suhtest ning nende eelnevast kokkusegamisest.

Parim lilla värvusega positiivne Weisneri reaktsioon saavutati 1:1 floroglütsinooli/HCl suhtega ning korralikult segatud suspensiooniga (reaktsiooniajaga 5 minutit) nii haavapuidus kui ka BCTMP-s. See, kas floroglütsinool ja HCl oli enne proovile kokkusegatud või mitte, ei andnud värvireaktsioonis erinevust. Selgelt oli ka näha, et proovi koguse suurendamisega kasvab ka värvi intensiivsus suspensioonis. Haavapuidu suspensiooni värvi intensiivsus oli tugevam kui BCTMP puhul. Selle meetodiga on võimalik näha kuni 5 % ligniini sisalduse vahet.

Punane intensiivne värvus tekkis Mäule reaktsiooniga nii haavapuidu kui ka BCTMP 0,1 g massiga proovides. Algul töödeldi proove 10 minutit 1% $KMnO_4$ lahusega kuni pruuni värvuse tekkimiseni, seejärel pesti destilleeritud veega. Edasi töödeldi kiudusid 3% HCl lahusega kuni pruun värvus muutus beežiks/kollakaks ja jälle pesti destilleeritud veega. Lõpuks töödeldi proove 2 minutit NH_4OH vesilahusega, mis muutis nii haavapuidu kui ka BCTMP proovid üsna sarnase intensiivsusega punast värvi. Seega on antud meetodiga võimalik hästi tõestada ligniini olemasolu, kuigi ligniini koguse visuaalne eristamine on keeruline.

Käesoleva töö uudsus seisneb selles, et kuigi värvireaktsioone on ligniini määramiseks puidus kasutatud alates 1900. aastast, ei ole seda siiamaani kvantiseeritud. Antud töös kalibreeriti värvireaktsioonide ligniini sisaldus UV ja FTIR instrumentaalsete meetodite abil. Senini on värvireaktsioone kasutatud peamiselt ligniini visuaalseks määramiseks *in situ* puidu struktuuris valgusmikroskoobis, kuid antud töös leiti, et värvireaktsioonide abil on võimalik visuaalselt kiiresti tuvastada ka jääkligniini olemasolu puitmassis.

Meetodi edasiseks arendamiseks tuleks tähelepanu pöörata ligniini väiksemate koguste määramisele. Kuigi Mäule testi polnud võimalik otseselt kasutada ligniini kalibreerimiseks, on see oluliselt tundlikum lehtpuidu jääkligniini suhtes. Teiseks tuleks instrumentaalanalüüsis kasutusele võtta UV - dispersioon Raman spektromeeter ligniini ning FTIR - Raman spektromeeter tselluloosi määramise jaoks.

Töö tulemusena saavutati püstitatud eesmärgid. Leiti lihtne meetod, mille abil on võimalik tuvastada kvantitatiivselt jääkligniini olemasolu haava puitmassis. Weisneri ja Mäule reaktsioonid toimisid puitmassis ning nende abil on võimalik jääkligniini määrata. Instrumentaalne ligniini kalibreerimine UV ja FTIR spektromeetri abil õnnestus hästi.