AN ABSTRACT OF THE THESIS OF

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Title: CHARACTERIZATION OF DISSOLVED WOOD ORGANICS IN TOTALLY-CHLORINE-FREE BLEACHING KRAFT PULP MILL EFFLUENTS

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Murray L. Laver

Six filtrate samples (liquid state) were obtained from selected stages in a totally-chlorine-free (TCF) bleach pulp mill. The solids were recovered by evaporation on rotary evaporators and by lyophilization with a freeze drier.

Chemical contents (inorganic and organic chemicals) of the six filtrates were analyzed quantitatively according to TAPPI Test Methods, 1988. Results showed that the #1BS filtrate had the largest amount of inorganic and organic chemicals, which would influence the pulp mill systems the most.

Thin-layer chromatography (TLC) was utilized to qualitatively analyze carbohydrates by using pre-coated cellulose plates. No free monosaccharides were detected in the six filtrates as received. The solids were then acid hydrolyzed and the monosaccharides, D-Glucose, D-Galactose, D-Mannose, D-Xylose, and L-Arabinose were detected in the hydrolyzates of the solids recovered from the filtrates of the early stages of bleaching. This showed that these samples contained both cellulose and hemicelluloses.

Wood extractives were also qualitatively determined by TLC using pre-coated silica plates. The freeze-dried solids were extracted with methanol, with distilled water and with ethyl acetate. Results revealed that dihydroquercetin, quercetin and catechin existed in the filtrates from the early stages of bleaching. These catechol-type chemicals will influence the problem of metallic accumulation severely.

The results of both carbohydrates and wood extractives were supported by Rf values, Rs (relative resolution) and Rx (relative retention).

Pulsed Fourier Transform carbon-13 nuclear magnetic resonance (NMR) spectroscopy was applied to study the functional groups, particularly hydroxyl groups, of the filtrate lignin samples with a proton decoupling method. A commercial kraft softwood lignin, Aldrich lignin, was used as a reference for the TCF mill filtrate lignins.

A newly emerged method of lignin acetylation with acetyl chloride enriched with carbon-13 as reagent was introduced. The conventional method of using acetic anhydride to acetylate lignin was used as a reference method. NMR spectra showed that the conventional method gave more functional group information, but the method of acetyl chloride acetylation determined the hydroxyl groups with greater resolution and higher speed. The TCF filtrate lignins were different from the commercial Aldrich lignin. Some differences were also found among the TCF filtrate lignins. These may be important for the future research of lignin modeling with metal complexity.

KEY WORDS: carbon-13 nuclear magnetic resonance spectroscopy (^{13}C-NMR), thin-layer chromatography (TLC), totally-chlorine-free (TCF), wood organics, bleach effluents, kraft pulp, pulp mill

CHARACTERIZATION OF DISSOLVED WOOD ORGANICS IN TOTALLY-CHLORINE-FREE BLEACHING KRAFT PULP MILL EFFLUENTS

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I. INTRODUCTION

This research is a part of a larger project dealing with non-process elements and organic compounds in pulp mills. The overall objective of the large project is to develop a fundamental, experimentally based method for predicting the solubility of organic and inorganic matters and their interaction in recycled effluents from bleach plants connected to kraft pulp mills.

The objective of this research is to characterize the dissolved organic chemicals in different stages of effluents (for convenience, they are called bleach filtrates) in a totally-chlorine-free (TCF) bleaching kraft pulp mill.

Historically, the pulp and paper industry is one of the largest consumers of water and so one of the largest producers of wastewater. As the environment problems become more and more important, the treatment of wastewater is becoming more and more urgent. Reducing the discharge of wastewater from pulping and bleaching operations is one of the most effective ways to improve environmental conditions near pulp and paper mills. To do so, the recovery and reuse of bleach plant filtrates is necessary. This creates a potential problem of accumulation of both organic matter (both volatile and non-volatile), and inorganic "non-process" elements such as barium, calcium, magnesium, aluminum, silica and heavy metals in the recycled water streams. This kind of accumulation will increase deposit formation and corrosion in digesters, pulp washers, recovery equipment and paper machines, and also increase the release of volatile organic matters to the environment. To solve this accumulation problem, an

understanding of the composition of the dissolved organic matters in the bleach effluents is needed (Frederick and Grace 1979, 1981; Westervelt 1981; Wilson 1987).

Wood organic chemicals cover a wide range of chemicals. Most of them are carbohydrates, lignin and wood extractives. Some of them are degraded by the harsh chemical conditions during pulping and bleaching, and are later dissolved in the liquor. They are capable of forming complexes with metal ions, which exist in pulping stages as cations, therefore increasing the total concentration of the metals in solution and causing deposition, corrosion and fouling problems (Wilson 1987).

The characteristic of organic matter that most strongly determines its ability to complex metal ions is the type and distribution of functional groups. Hydroxyl and carboxyl groups are the predominant functional groups in dissolved wood organic chemicals. These anionic groups can complex with many metal ions (Frederick and Grace 1979; 1981).

II. LITERATURE REVIEW

A. Dissolved Wood Organic Chemicals

Wood organic chemicals cover a wide range of chemicals. Most of them are polysaccharides, lignin and wood extractives (see figure 2.1) (Browning 1967). Actually, some free monosaccharides and oligosaccharides may also be present in wood (Sjostrom 1993). When in harsh chemical conditions during pulping and bleaching, these organic chemicals may be degraded partly or totally due to their active functional groups. Part of these derivatives and non-derivatives will still be absorbed to pulps physically or chemically, whereas others will be dissolved and flow with the spent pulping liquors (Casey 1980; Singh 1979; Hough 1985; Sjostrom 1993).

The dissolved organic chemical compositions of kraft black liquors have been analyzed very well during the past years. They mainly consists of lignin, carbohydrate degradation products, turpentine and tall oil (resin and fatty acids). Table 2.1 shows the chemicals in black liquors (Hough 1985).

Usually, the turpentine and the tall oil fractions are recovered because of their considerable by-product value (Sjostrom 1993). Lignin and carbohydrate degradation products in the black liquors contain chemical functional groups that are anionic and active to react with the non-process metal elements. This creates a problem of accumulation of both organic and inorganic chemicals and so increases deposit formation and corrosion (Westervelt 1979; Frederick and Grace 1979, 1981). Recent work by Wilson (1987) involved research of the interactions between the dissolved wood organic chemicals and the non-process elements in kraft black liquors.

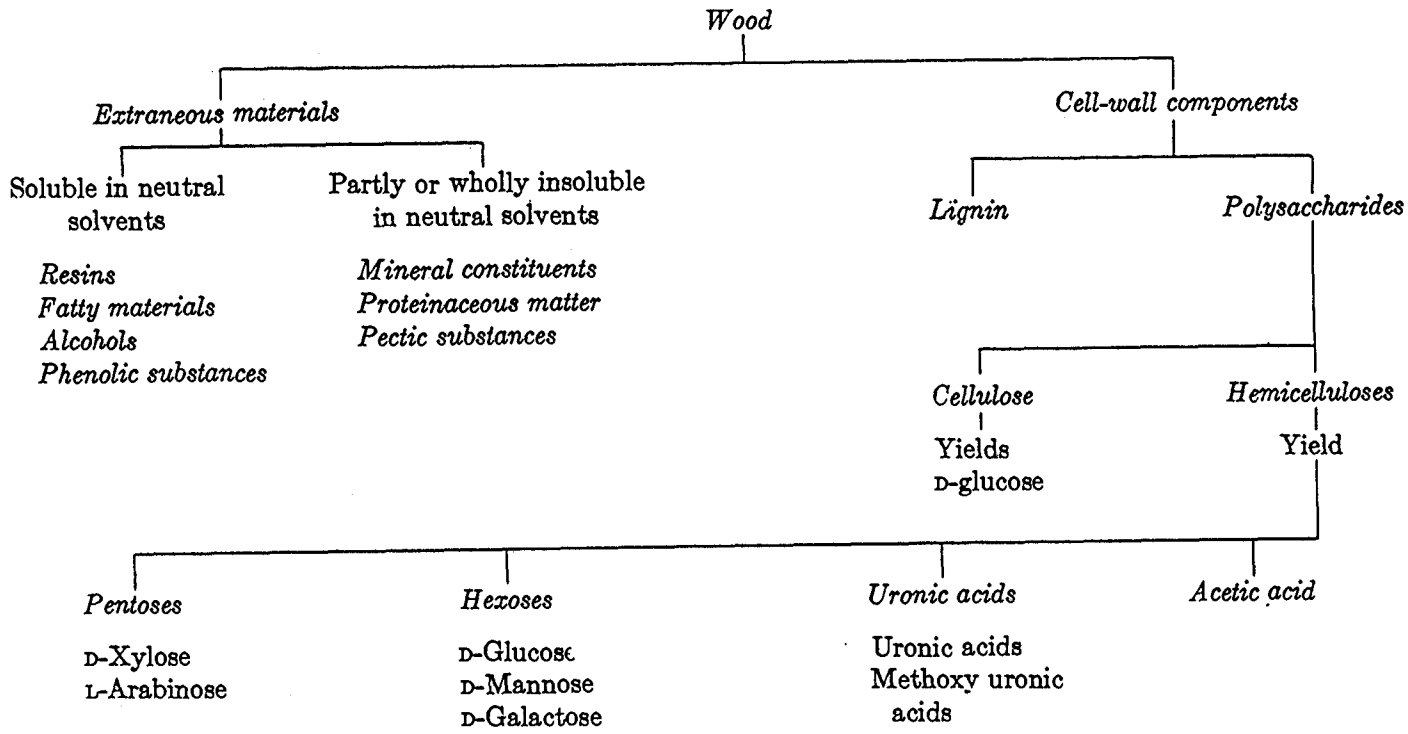

Figure 2.1. The composition of wood (Browning 1967).

Table 2.1 Chemical components in black liquors
(Expressed as percentage of dry solids) (Hough 1985)

Wood	Pine	Pine	Spruce
Dry solids in sample, %	17	58	
Lignin	28.9	30.70	41
Hemicellulose and sugars	1.14	0.11	
Extractives	6.69	2.53	
Saccharinic acids			28
Acetic acid	3.52	2.08	5
Formic acid	4.48	2.7	3
Other organic acids	5.5	2.22	
Methanol			1
Unknown organic compounds	19.0	29.5	
Inorganic salts	18.6	18.5	
Organically combined Na	10.1	10.3	
Unknown inorganic compounds	2.08	1.35	
Sulphur, S			3
Sodium, Na			16
Total	100	100	100

Black liquors are those spent pulp liquors that are collected from the digestor after alkaline pulping. In the kraft pulp mill where we obtained samples, there were two stages of brown stock washing, and the washing filtrates were stored in respective seal tanks before they were mixed and treated with the kraft black liquors. So far, no research related to the brown stock filtrates has been found. Part of this study is to answer whether the black liquors are the same as the brown stock filtrates.

There has been no work related to the determination of the chemical composition in totally-chlorine-free (TCF) bleaching filtrates. Because of the reaction of oxygen-containing bleaching reagents (oxygen, hydrogen peroxide and ozone) with kraft pulps, there should be some differences in the TCF bleaching filtrates.

B. Totally-Chlorine-Free (TCF) Bleaching

The purpose of chemical pulping is to selectively remove the fiber-bonding lignin to some degree while keeping as much as possible the cellulose and hemicelluloses. Not all of the lignin is removed because otherwise the yield and the strength of the chemical pulps would be reduced excessively. Therefore, the bleaching process is required to remove the remaining lignin and its degradation products in order to obtain high-brightness pulps (Casey 1980). Also, some of the other chemical components, including carbohydrates and their degradation products, resins, metal ions and other compounds of various kinds have to be removed or modified for whiter, brighter and stable pulps (Singh 1979).

As an environment-friendly method compared with the conventional chlorine bleaching process, the totally-chlorine-free (TCF) bleaching process is becoming more and more important (Gierer 1997; Gierer and Imsgard 1975; Kadla et al. 1997; Mielisch et al. 1995; Richard 1994; Casey 1980).

So far, most works have been focused on the reaction mechanisms of the pulps with the common oxygen-containing bleaching reagents (oxygen, hydrogen peroxide and ozone). Due to the variable properties of pulps, and the great variety in the structures of the organic substrates, many model compounds for lignins and carbohydrates have been introduced (Singh 1979; Gierer 1997, 1985; Gierer and Imsgard 1975; Kadla et al. 1997; Mielisch et al. 1995). However, the understanding of the reactions is still far from being complete, because the reaction in oxygen bleaching is related with those reactions in hydrogen peroxide and ozone bleaching due to "the participation of common reagents (oxygen and hydrogen peroxide) and common

intermediary radical species (superoxide/hydroperoxyl and hydroxyl radicals) arising from these reagents" in all the three bleaching processes (Gierer 1997).

No matter how extensive the products are, there are two types of oxidation reactions with lignin: a) Nucleophilic addition and displacement reactions and b) Electrophilic addition and displacement reactions (see Figure 2.2) (Gierer 1982).

From this figure, we can determine that all the hydroxyl groups in the lignin, primary hydroxyl, secondary hydroxyl and phenolic hydroxyl, have the possibility to be reacted with the TCF bleaching reagents. However, no one can tell which hydroxyl group is more likely to react. In this work, we try to answer this question by analyses of the lignin in TCF bleaching filtrates.

For reactions with carbohydrates in TCF bleaching processes, it was said the above reaction mechanism for lignin is also valid for carbohydrates, and that is the main cause of the limited selectivity of TCF bleaching (Gierer 1982; Gierer 1997).

For reactions with wood extractives, Casey (1980) concluded that the content of extractives in pulps would drop to a very low level if multistage bleaching was used after a stage of oxygen bleaching.

Recent work by Ristolainen et al. (1996) involved determination of average molecular weights and molecular-weight distributions of hardwood lignin dissolved in various stages of ozone bleaching effluents by using gel permeation chromatography (GPC) and ultrafiltration (UF). The results are shown in Table 2.2. It seemed that the average molecular weights of lignins after the O, EOP and EP stage were all larger than 1000, and the molecular-weight distributions of them were all larger than 60%. These results were referred to our research so that we could use 1000 of MWCO of dialysis

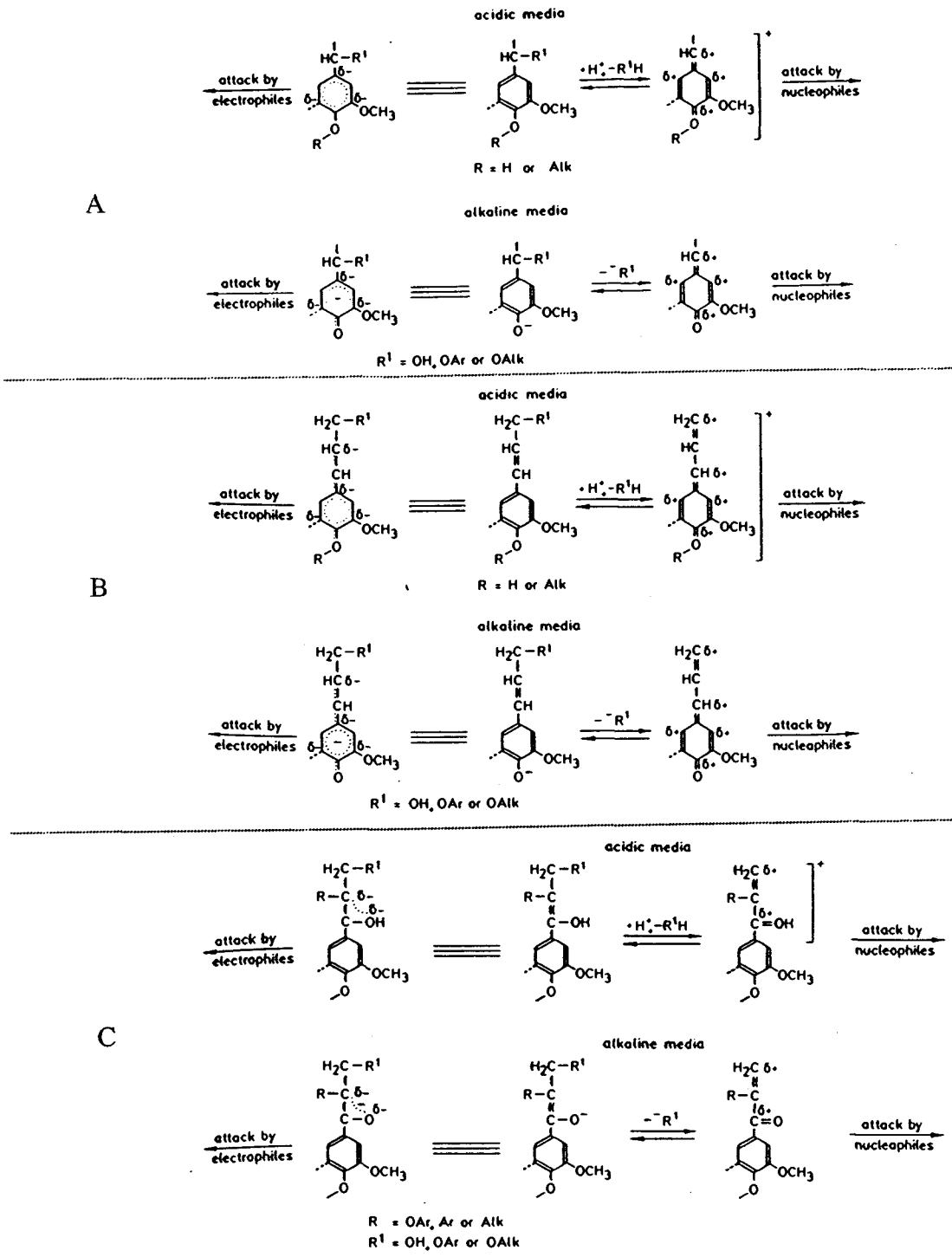

Figure 2.2. Sites of electrophilic (δ-) and nucleophilic (δ+) attack in lignin units. (A: Arylalkane units; B: Arylpropene units; C: Units containing an α-carbonyl group) (Gierer 1982)

Table 2.2. Average molecular weight (Mw) and molecular-wight distribution (Md) for lignins in the Ozone Bleaching Effluents [Data from (Ristolainen 1996)]

Effluent	O	X	Z	EOP	A	EP	S
Mw (a)	5352	3284	1391	3328	730	2305	2314
Mw (b)	4812	2913	1473	3443	906	2022	2128
Md (<1,000)	39.9	29.5	52.1	25.3	73.3	35.1	44.7
Md (>1,000)	60.1	70.5	47.9	74.7	26.7	64.9	55.3

Notes: 1. O--oxygen/alkali; X--xylanase treatment; Z--ozone; EOP--alkaline extraction with oxygen and peroxide; A--acid treatment with small ozone addition; EP--alkaline extraction with peroxide; and S--acid souring with sulphur dioxide.
2. (a)--under 245 nm ultra violet (UV) microscopy; (b)--under 280 nm UV.
3. (<1000)-- Mw less than 1000; (>1000)--Mw more than 1000.

membrane to purify the lignins in our TCF (oxygen and hydrogen peroxide as reagents) bleaching fitrate samples without worrying about losing too many lignins.

C. Thin-Layer Chromatography (TLC)

As a separation technique, thin-layer chromatography (TLC) was first introduced in 1938 by two Russian workers, Izmailov and Schraiber. However, little notice was made of the method in its early years because there were no available media and apparatus to coat the plates lacked uniformly. The effectiveness of the technique for separation was shown when Stahl described equipment and efficient sorbents for the preparation of plates in 1958. Now the method is one of the most widely used separation techniques in chemistry (Touchstone 1992; Hamilton and S. Hamilton 1987).

Compared with gas chromatography and liquid chromatography, thin-layer chromatography has a lot of advantages, such as ease of use (easy change of mobile

phase), wide application to a great number of different samples, speed of separation, low cost and handling lots of different samples at one time (Touchstone 1992).

Results of TLC can be better analyzed by using the R_f value, relative resolution (Rs) and relative retention value (Rx). The success or not of a separation of compounds can be identified by Rs, which should be not less than a unit. R_f value is the characterstic of a chemical (Touchstone 1992; Hamilton and S. Hamilton 1987).

Usually three types of sorbent materials in thin layer are used to coat on plates, silica gel, alumina and cellulose. Cellulose plates are used to separate hydrophilic compounds such as sugars, which can migrate due to less bonding energy with the cellulose molecule than with alumina or silica gel (Hamilton and S. Hamilton 1987).

Silica gel plates were found to separate wood extractives extracted from Douglas-fir stained and unstained sawn lumber by using methanol (Laver and Wang 1996). Their results showed that dihydroquercetin was the major contributor to the color development and the most important component of chemical brown stain discoloration. Catechin and epicatechin were also found to exist. All of these three extractives have the catechol group. Catechol compounds are known to chelate with many metal ions in aqueous solution and so contribute to the accumulation problem (Westervelt 1979).

In this work, cellulose and silica gel pre-coated plates were used to characterize the carbohydrates and extractives respectively in the brown stock filtrates and the TCF bleaching filtrates. A technique of multiple development was tried. This method was first applied to TLC in 1955 by Mottier and Potterat. The main reason to do so is to increase the effective length of the layer so that all the components can be separated

reasonably and effectively (Touchstone 1992). Two-dimensional cellulose was also tried according to the work of Karchesy et al. (1989).

D. Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR)

Carbon-13 NMR spectrometry has been used for lignin structural analyses for many years. It is a non-destructive technique, not like lignin analyses using chemical methods that often involve the lignin and its degradation products in structural changes. Its spectrum gives more structural information in a wider chemical shift range than pronton-1 (^{1}H) NMR because carbon forms the backbone of all organic chemicals (Robert 1992; Field and Sternhell 1989).

For quantitative analyses of lignin and its acetates, pulsed fourier transform (PFT) ^{13}C-NMR spectroscopy usually was used with 90° flip angle and 10-12 seconds pulse delay (Robert 1992; Labidi et al. 1993; Faix 1994; Pan and Lachenal 1994; Robert and Brunow 1984; Orejuela and Helm 1996). There were also other conditions, such as 30° flip angle and 1.0 second pulse delay (Morck et al. 1988), 20° flip angle and 1.0 second pulse delay (Morck and Kringstad 1985), and 20° flip angle and 0.2 second pulse delay (Krinstad and Morck 1983). The 90° pulse is preferred because it yields the highest signal intensity (Robert 1992).

Hexadeuteriated dimethylsulfoxide (DMSO-d6) was used in all the above references. It is widely used because no significant lignin signals overlap with its ^{13}C signal at 39.5 ppm of chemical shift (Robert 1992).

However, the above conventional NMR is very time-consuming and needs a lot of scans for a good signal-to-noise ratio (S/N). Pan (1994) used 7,500-10,000 scans

(over 24 hours), while other workers used more than that. Also, large samples were needed for good results. Pan (1994) used 250-400 mg of lignin samples. Robert (1992) proposed that 400-600 mg of lignin samples was suitable, and at least 100 mg should be needed. For natural compounds such as lignin, this is not very favorable because it means that a large weight scale of raw material is needed to produce so much pure natural compound.

To avoid these problems, a very new method was proposed by Orejuela and Helm (1996) for lignin analysis. They used [1-^{13}C]-acetyl chloride instead of the conventional acetic anhydride (referred to in the above references) to acetylate lignins so that the time of running NMR was shortened to only about 15 minutes (64 scans) and the amount of lignin used was only 10-50 mg. The drawback of this method seemed to be that it could only provide lignin structural information at 160-175 ppm of the chemical shift range, where hydroxyl groups are assigned, because the workers showed the spectra only at this range. No other references using this method were found.

None of the above works dealt with TCF bleaching filtrates. Most of them dealt with kraft lignin from the kraft pulping process (Wilson et al. 1996; Labidi et al. 1993; Gellerstedt and Robert 1987; Morck et al. 1988; Morck and Kringstad 1985; Krinstad and Morck 1983). Some works included research on kraft lignin (Faix 1994; Orejuela and Helm 1996). In this work, both acetylation reagents (acetic anhydride and [1-^{13}C]-acetyl chloride) were tried to find out the differences in lignin structure in various stages of filtrates (including brown stock filtrates and TCF bleaching filtrates). Due to a lack of samples, only about 20-80 mg of each sample was used for each method. Results were compared with the results from the above references.

III. EXPERIMENTAL

The processes and experiments for the filtrate samples are shown in Figure 3.1.

A. Description of Samples

Six filtrate samples capped in plastic bottles were obtained from different stages in the Louisiana--Pacific Corporation, Samoa, CA pulp mill. It produces totally-chlorine-free (TCF) bleached kraft market pulp continuously by using hydrogen peroxide (H_2O_2) and oxygen (O_2). The samples were kept at room temperature. Because they came from different stages where different conditions were applied, they had different colors, odors, appearances, and different pH values. According to the names of these stages, they were called: #1BS Filtrate, CB Filtrate, PO_2 Filtrate, Q Filtrate, EOP Filtrate and P3 Filtrate.

Though our main interest was in these filtrates, six different pulp samples were also obtained from different pulping and bleaching stages in the same mill in order to have some comparison with the filtrate samples. They were kept in a refrigerator at about 4 °C, and called: #2BS pulp, Q pulp, EOP pulp, P1 pulp, P2 pulp and P3 pulp.

The process of the TCF bleaching in this mill is shown as Figure 3.2, which also shows where the filtrate and pulp samples were collected.

B. Chemical Contents Analysis

The inorganic and organic chemical contents of the six filtrate samples were determined by analyzing the solids content (SC), dissolved solids content (DSC), suspended solids content (SSC), ash content (AC), moisture content (MC) and Klason lignin content (KLC) according to TAPPI Test Methods, 1988. The SC, DSC and SSC

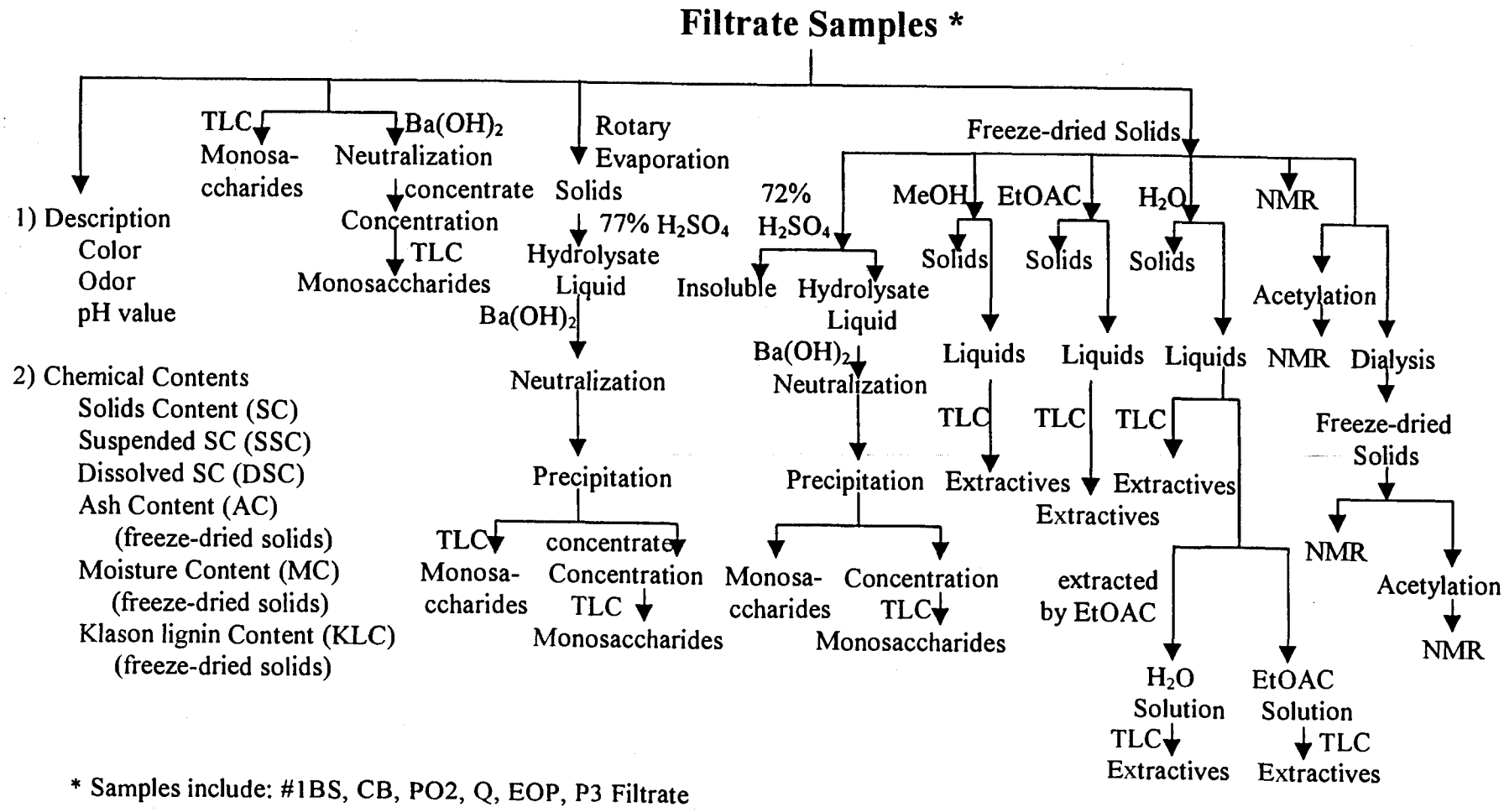

Figure 3.1. Processes and experiments for the TCF filtrate samples in this work

(samples were from Louisiana-Pacific Corp., Samoa, CA Pulp Mill)

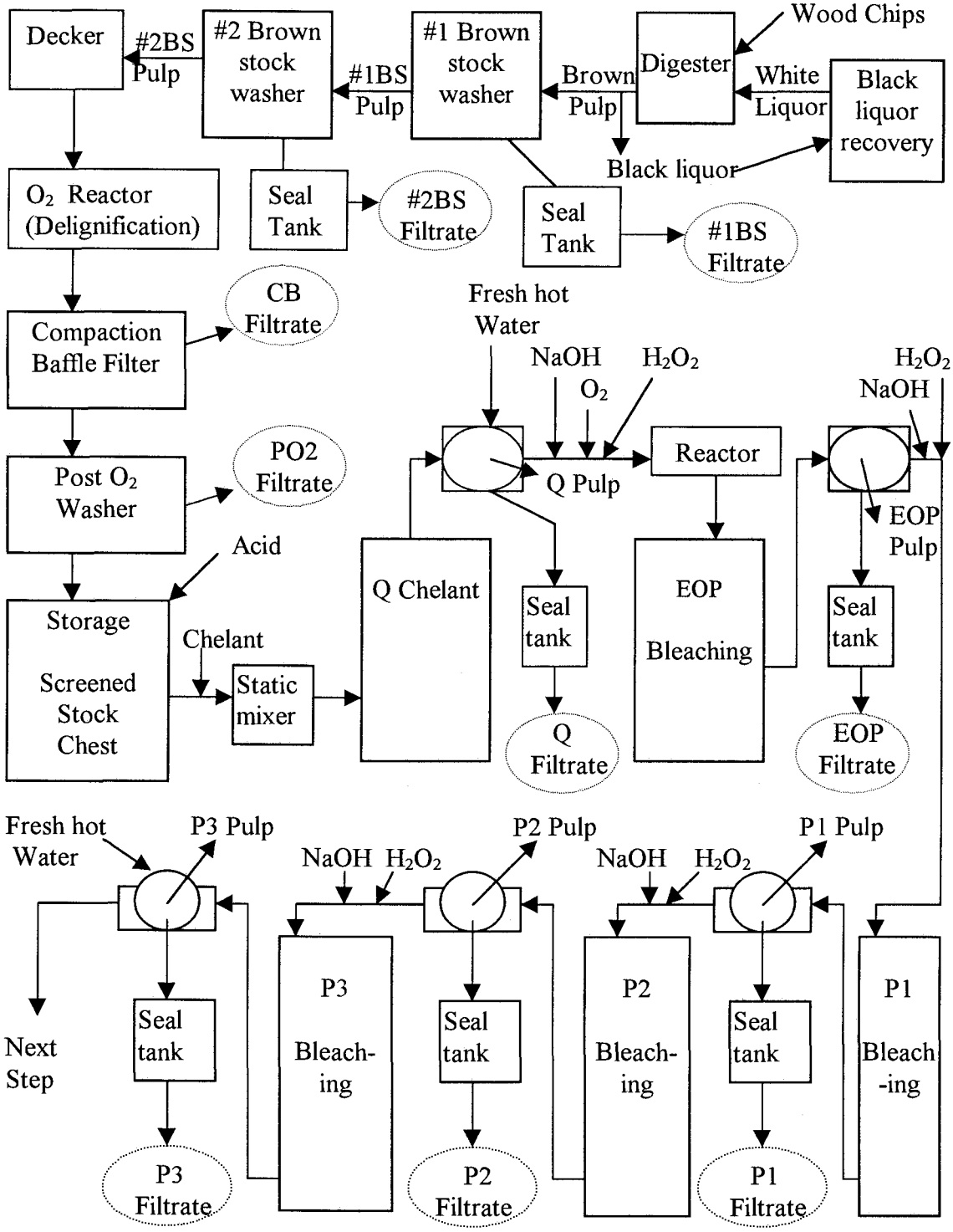

Figure 3.2. Simple drawing of TCF bleaching processes in the pulp mill

(Derived from Louisiana--Pacific Corp., Samoa, CA pulp Mill)

contents were determined by TAPPI T650 pm-84, which basically involved drying the samples in a forced-air oven at 105 ± 3 °C.

The AC content was determined by TAPPI T211 om-85, which involved ashing the sample in a muffle furnace at 575 ± 25 °C. The MC content was determined by TAPPI T264 om-87 by drying to constant weight at 105 ± 3 °C. The KLC content was determined by TAPPI T222 om-83 by first dissolving the sample in a 72% sulfuric acid, then diluting to 3% sulfuric acid followed by reflux. The insoluble fraction was recovered by filtration, dried and weighed as Klason lignin.

For SC determination, the test specimens were the six filtrate samples as received. They were stirred before using so that the suspended solids were uniformly distributed in the solutions.

A centrifuge (Ivan Sorvall Inc., USA) was used at 7500 RPM to separate the filtrate sample into two kinds of materials in a centrifuge tube: the upper liquid, which contained dissolved chemicals, and the lower wet solids, which contained suspended chemicals and fibers. The upper liquid was then used as the test specimen to determine the DSC in the filtrate sample. The lower wet solid material was the test specimen to determine the SSC in the filtrate sample.

Some volumes of the six filtrate samples were freeze-dried for about 6 days to almost non-moisture solids by using a freeze-drying machine (FTS® SYSTEMS INC.). Then the freeze-dried solids were kept in closed glass bottles to be used as test specimens in the following AC, MC and KLC determinations, thin layer chromatography (TLC) analysis, and nuclear magnetic resonance (NMR) analysis.

C. Thin-Layer Chromatography (TLC)

A general process of thin-layer chromatography used in this work is presented in Figure 3.3 (Touchstone 1992). The equipment needed is described in Figure 3.4 and consists of a stationary phase system (a thin layer of absorbents on a plate), a mobile phase system (developing solvent system), and a developing tank with lid.

C.1 Characterization of Monosaccharides by TLC

Monosaccharides are simple sugars, of which D-glucose, D-mannose, D-galactose, D-xylose, and L-arabinose are the most common constitutes of the cell wall polysaccharides in wood. We can thus use these five authentic monosaccharides as our standards when using TLC to characterize our filtrate and pulp samples as Douglas-fir wood chips were the main original materials for the pulp mill (see Figure 3.5).

Cellulose pre-coated plates (G1440 Cellulose, 5×20 cm, Schleicher & Schull GmbH, W.Germany) were used to perform this experiment. The technique of multiple development (developed three times) in one dimension was used in this experiment: Develop→ Air dry→ Develop→ Air dry→ Develop→ Air dry.

The developing solvent used in this work was ethyl acetate-pyridine-distilled water (EPW 8:2:1 v/v). After developing, the plate was sprayed with aniline hydrogen phthalate reagent (1.66 g of phthalic acid dissolved in 100 ml of water-saturated n-butanol containing 0.93 g of freshly distilled aniline). Then after the plate had been air-dried for about 10 minutes, it was heated in an oven at 105 °C to 110 °C for 10 minutes.

The filtrate samples were spotted onto the plates by using 1.0 mm capillary tubes. The samples were analyzed as received and also after treatments that included hydrolysis,

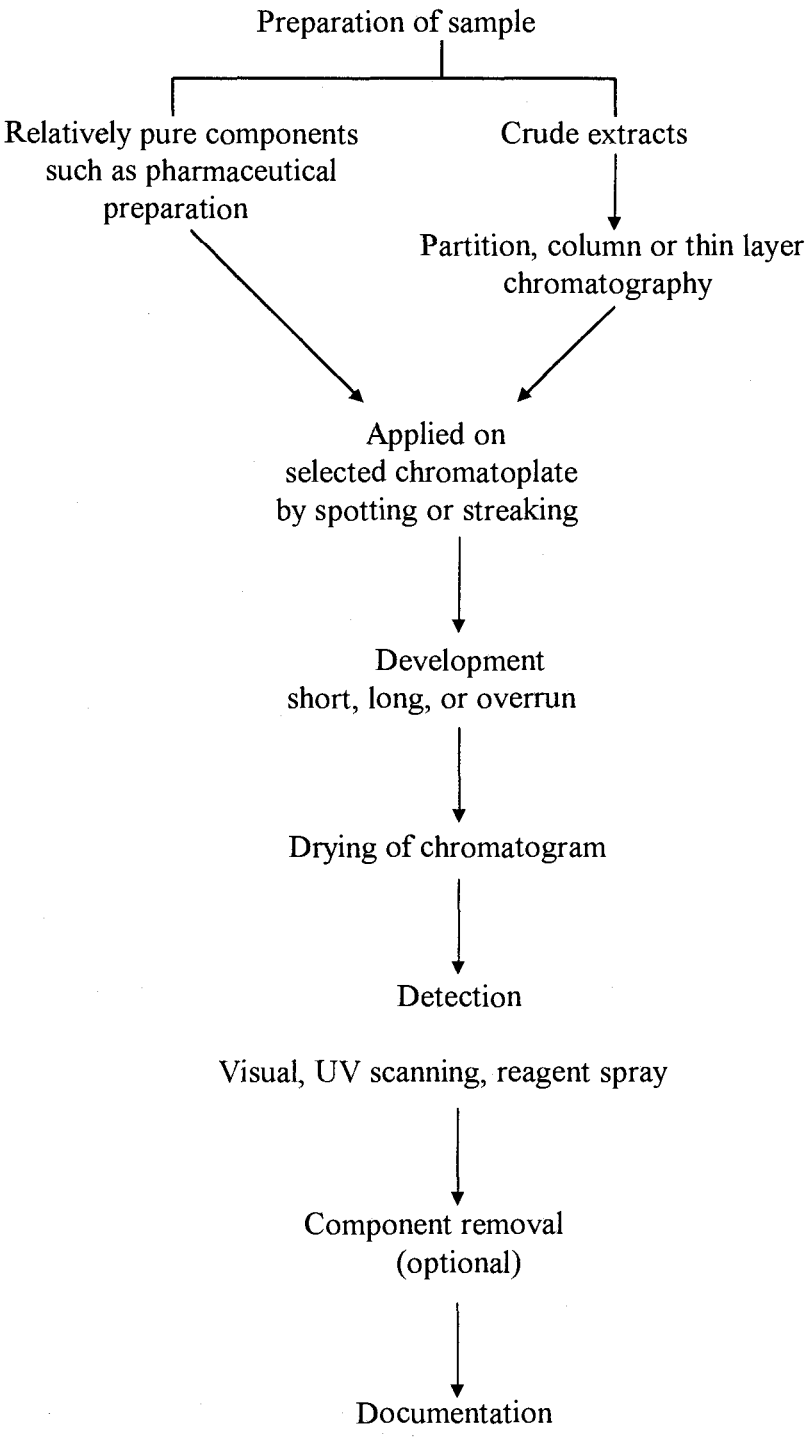

Figure 3.3. A general process for thin-layer chromatography

(Touchstone 1992)

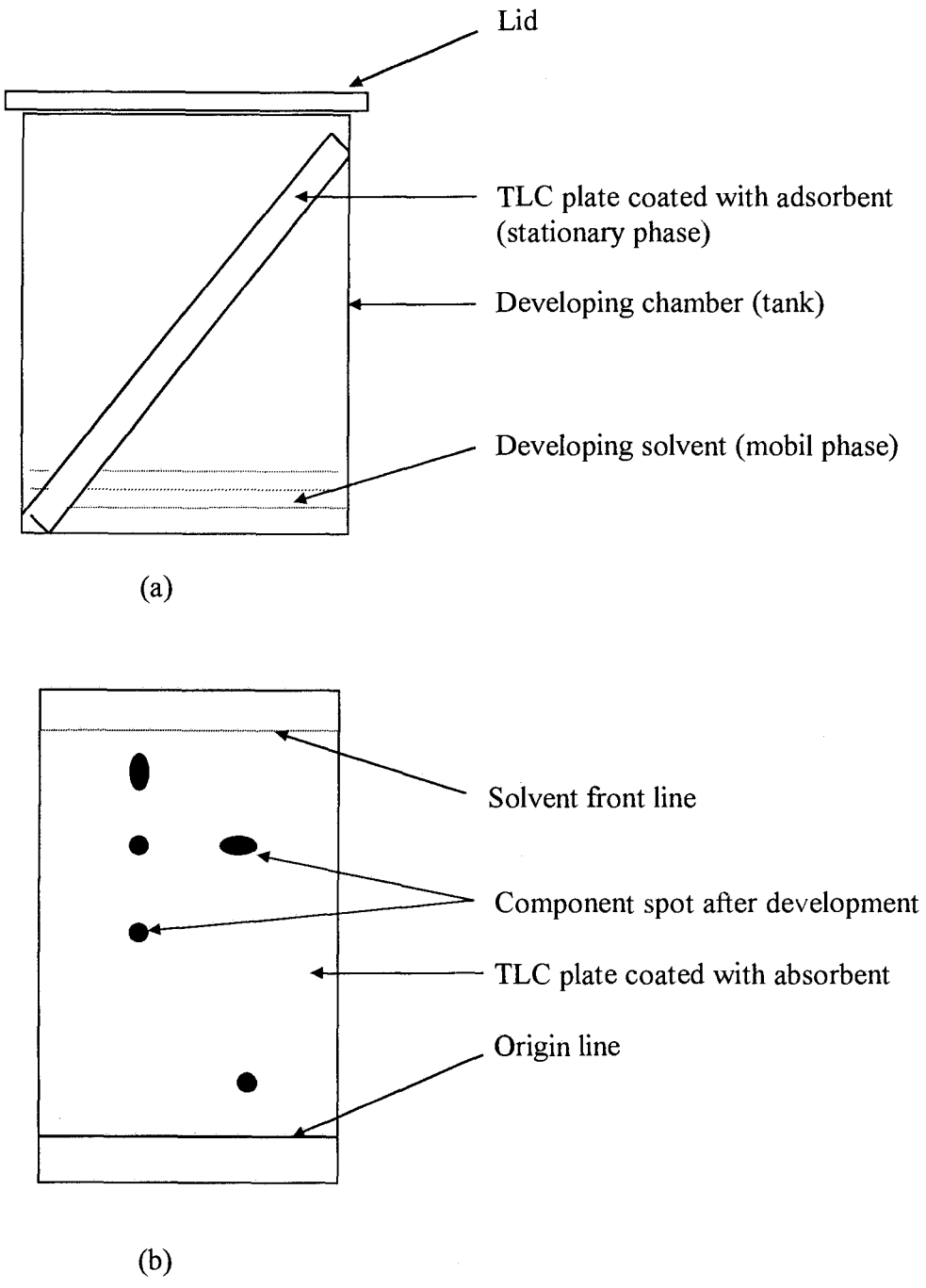

Figure 3.4. The main equipment of thin-layer chromatography

(a) The total systems in TLC ; (b) The detailed plate after TLC

neutralization and concentration. Hydrolysis was done by using 72.0% and 77.0% sulfuric acid. Neutralization was performed by adding calcium hydroxide [$Ca(OH)_2$] to pH 6-7. Concentration was done by using a rotary evaporator.

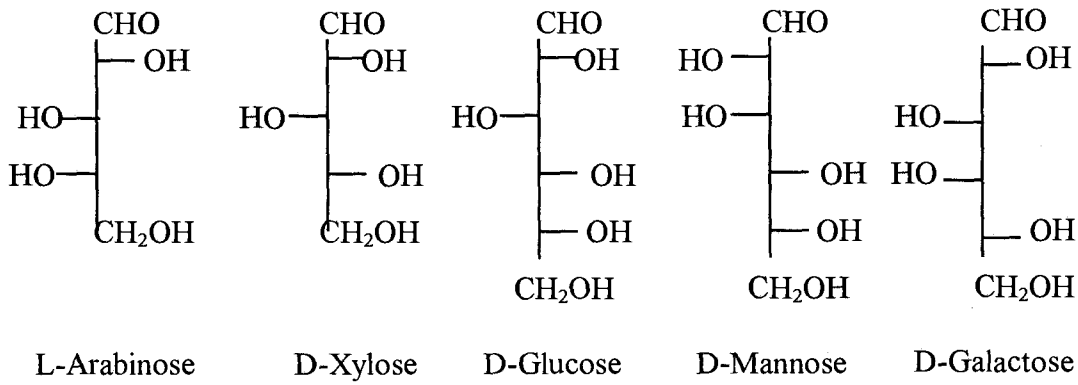

Figure 3.5. Structures of the monosaccharides used as standards in TLC

The procedure for hydrolysis with 77.0% sulfuric acid was as follows: transfer 5.0 ml of 77.0% sulfuric acid with a pipette into a 250 ml round-bottomed flask submerged in an ice bath. Transfer enough sample to the round-bottomed flask to ensure 0.21 g (approximately) of dried solids. Mix the sample with the sulfuric acid for 5 minutes in the flask in the ice bath by using a glass rod. Remove the flask from the ice bath and let it come to room temperature for about 2 hours. Add 123.0 ml of water dropwise with the sample flask was submerged in the ice bath. Reflux the solution in the round-bottomed flask for 5 hours at 80 °C. Take away the flask, and dilute the hydrolysate to 250.0 ml in a volumetric flask. After neutralization, the hydrolysate may or not be concentrated before TLC was performed (Laver et al. 1967).

The procedure of hydrolysis with 72.0% sulfuric acid was the same as in the Klason lignin (KLC) determination.

C.2 Characterization of Wood Extractives by TLC

The wood extractives we were most interested in were quercetin, dihydroquercetin (taxifolin) and catechin. They all have catechol groups.

Authentic dihydroquercetin (DHQ), quercetin (Q) (both from K&K Laboratories, Inc.), and (+)-catechin (C) (Sigma Chemical Co.) were dissolved in methanol (MeOH, analytical grade) to prepare 0.25% solutions as the known standards of TLC.

Silica precoated plates (DC-plastikfolien Silica Gel 60 F254 with plastic support and DC-fertigplatten Silica Gel 60 F254 with glass support) were mostly used in this experiment. G1440 cellulose plates were also tried. All of them were cut into 5×10 cm pieces. Samples were spotted by using 0.5 mm diameter capillary tubes.

Different developing methods (1, 2 or 3 times of developing) in one dimension were tried. Two dimensional (2-D) cellulose TLC (10 ×10cm plate) was also tried.

After developing, the visualization steps were performed as follows: for cellulose plates, the spray reagent, vanillin-hydrochloric acid, was applied; for silica gel plates, the spots on the thin-layer plates were viewed in a Chromato-Vue box under short-wave (254 nm) ultraviolet light.

C.2.1. Sample Preparation

At first, methanol (MeOH, analytical grade), ethyl acetate (EtOAc, analytical grade) and distilled water were tried to extract the freeze-dried solids of #1BS Filtrate

and CB Filtrate. For the EtOAc extraction, distilled water was again used to repeat the extraction. All the extractives after extraction were detected by TLC. Then according to the results, only MeOH was used to extract the Q, EOP, and P3 filtrate solids. Different solubilities of the #1BS filtrate solids that dissolved in these three extractive solvents were also determined. The procedure of extraction was as following (Figure 3.6):

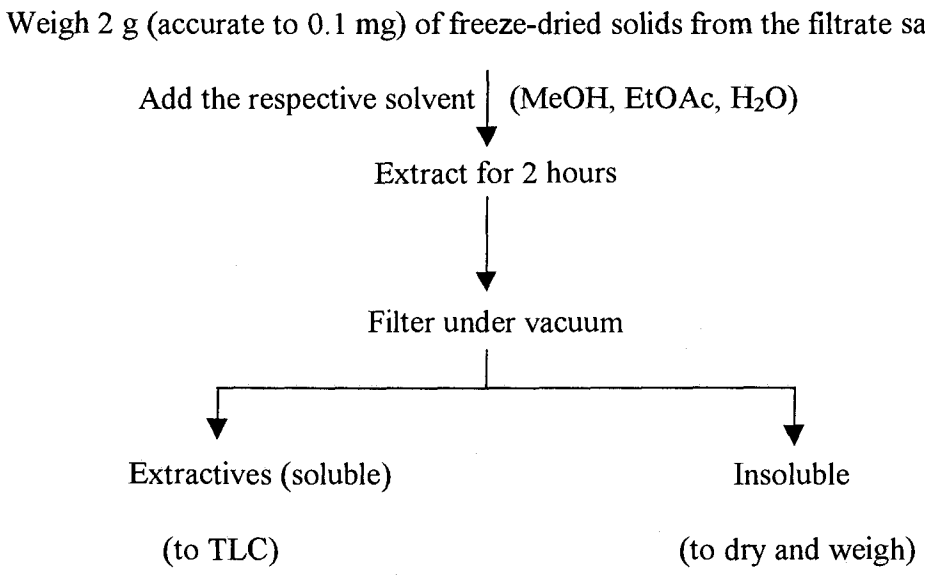

Figure 3.6. The process of extraction of filtrate sample

C.2.2. Selection of Developing Solvent System

Table 3.1 shows different chemical polarities (Touchstone 1992).

To find out the optimum developing solvent system, we tried a selection of chemicals according to their polarities (all ratios were v/v): Methanol (100%); Methanol-n-Hexane (1:1); Methanol-n-Hexane (1:3); Benzene-Acetone-Methanol (6:3:1);

Benzene-EtOAc (4:1); Chloroform-EtOAc-Methanol (3:1:1), (3:1:2), (2:1:2), (2:1:3) and (6:1:3); Chloroform-EtOAc-Formic acid --(5:4:1), (5:2:1) and (3:1:1); Toluene-Acetone-Formic acid (7:3:1), (5:3:1), (5:5:1), (3:3:1) and (3:5:1), 6% Acetic acid.

Table 3.1. Polarities (P') of different chemicals (Touchstone 1992)

Name	Polarity (P')	Name	Polarity (P')
Hexane	0.0	Ethanol	5.2
Triethylamine	1.8	Pyridine	5.3
Toluene	2.3	Acetone	5.4
Diphenyl ehter	2.8	Methoxyethanol	5.7
Diethyl ether	2.9	Aniline	6.2
Benzene	3.0	Methylformamide	6.2
Methylene chloride	3.4	Acetic acid	6.2
t-Butanol	3.9	Acetonitrile	6.2
Tetrahydrofuran	4.2	Dimethylformamide	6.4
Ethyl acetate	4.3	Dimethylsulfoxide	6.5
Chloroform	4.4	Methanol	6.6
Dioxane	4.8	Formamide	7.3

D. Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR)

The ^{13}C-NMR experiments were performed in 10 mm tubes with a Bruker@ AC300 and a Bruker@ AM400 instrument (Brucker Instruments, Incorporated, Manning Park, Billerica, Massachusattes 01821). For quantitative analysis, the standard proton-decoupled 1D ^{13}C pulse program was used with a 90° pulse and a relaxation delay of 12 seconds (see Table 3.2 for detailed information about the operation conditions). The integration ranges were determined both automatically (auto-phase correction program) or manually. Different scan numbers (NS) were used for different samples.

Two kinds of acetylation agents were used to acetylate lignin samples before ^{13}C-NMR analyses. One was acetic anhydride ($CH_3CO\text{-}O\text{-}OCCH_3$), the other was acetyl chloride ($CH_3{}^{13}COCl$). The general acetylation process is shown in Figure 3.7. After acetylation, the hexadeuterated dimethylsulfoxide (DMSO-d_6) was used as the solvent to dissolve the lignin acetates, and also as the internal reference with ^{13}C chemical shift at 39.5 ppm. When added to a 10 mm NMR tube, the solution of lignin acetates was filtered through a syringe plugged with glass wool to remove suspended impurities.

Table 3.2. Some important parameters used in the NMR experiments

Name	^1H operation frequency (MHZ)	^{13}C operation frequency (MHZ)	Pulse width (PW)	Relaxation delay (RD, s)	Acquisition time (AT, s)	Temp-erature (K)
AC300	300	75.5	7.5 (=90°)	12.00	1.769	297
AM400	400	100.6	8.9 (=90°)	12.00	1.311	298

D.1 Characterization of Aldrich Lignin by ^{13}C-NMR

Aldrich lignin is a commercial, softwood kraft lignin purchased from Aldrich Chemical Company. It was kept in a plastic bottle at room temperature.

The optimum lignin acetylation condition with acetic anhydride was chosen from the ^{13}C-NMR spectra obtained under the conditions shown in Table 3.3.

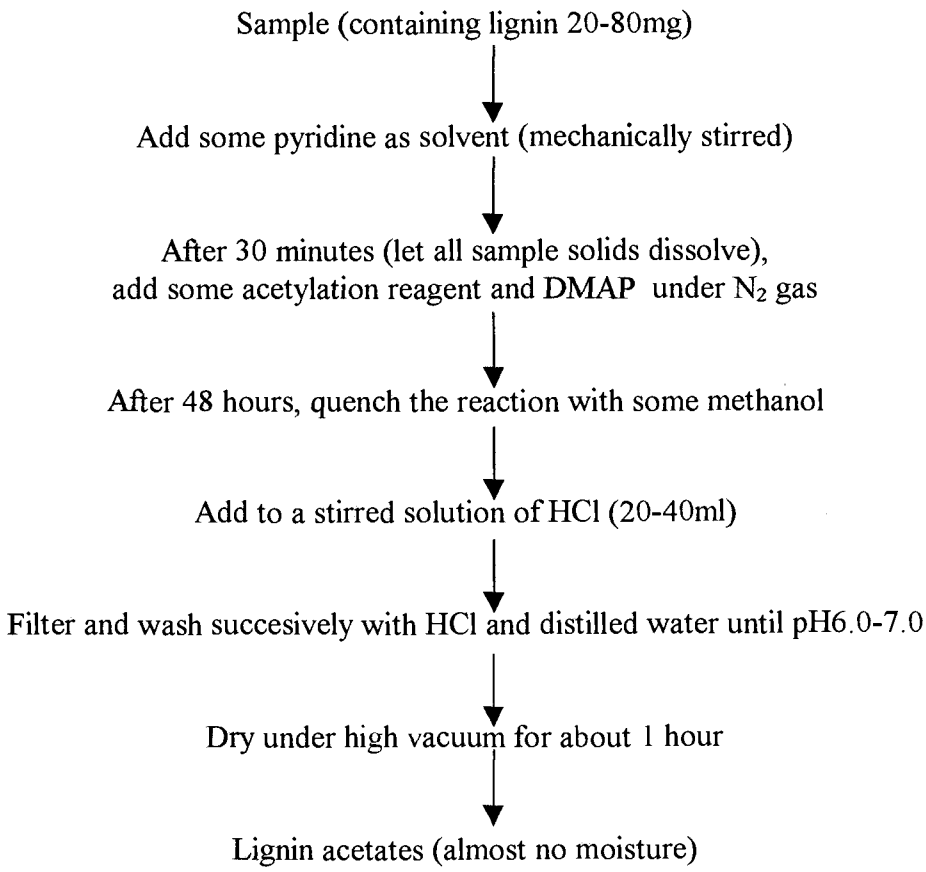

Figure 3.7. Process of lignin acetylation used in this work

(Derived from Orejuela and Helm 1996)

Two different acetylation conditions with $CH_3{}^{13}COCl$ were tried. The main difference between them was the order of adding the $CH_3{}^{13}COCl$ (as reaction reagent) and the 4-dimethylaminopyridine (DMAP) (as catalyst). Condition G was that $CH_3{}^{13}COCl$ was added first and then DMAP was added, while Condition H was that DMAP was added first and then $CH_3{}^{13}COCl$ was added.

Table 3.4 showed all the samples of Aldrich lignin analyzed by ^{13}C-NMR.

D.2 Characterization of Filtrate Lignin by ^{13}C-NMR

#1BS, #2BS (received later), CB, Q and PO2 filtrates were characterized. Because of the lack of enough samples, the amount of lignin contained in the Q and PO2 filtrate to be acetylated were both less than 10 mg.

The processes of using ^{13}C-NMR were similar with that for Aldrich lignin. However, these filtrates contained relatively low contents of lignin, and since they were directly collected from the pulp mill, they contained insoluble fractions, carbohydrates, wood extractives and other chemicals, which affected the experiments and made them difficult to analyze by NMR.

Dialysis filtration was used to purify these filtrates by eliminating those chemicals that might affect the ^{13}C-NMR analyses, including small sized carbohydrates and wood extractives. The dialysis filtration membrane was made from Spectra/Por ®, with a molecular weight cut off (MWCO) of 1000 and was kept in a 0.1% sodium azide solution in a refrigerator. It was 24 mm in diameter. Before using, the membrane was cleaned with distilled water. A simple setup of dialysis filtration is shown in Figure 3.8. After dialysis, the solution was freeze-dried. Then the freeze-dried solids were acetylated using the optimum conditions which were obtained as shown above for Aldrich lignin.

Figure 3.9 shows the sample preparation for ^{13}C-NMR of the #1BS filtrate. This sample preparation was typical of the ^{13}C-NMR analyses of other filtrates.

Table 3.5 lists all the filtrate samples that were analyzed by ^{13}C-NMR.

Table 3.3. Acetylation conditions of Aldrich lignin with acetic anhydride

Condition	Aldrich lignin (mg)	Pyridine (ml)	Acetic anhydride (ml)	Methanol (ml)	HCl (N)
A	49.27	3.0	1.0	2.0	1.2
B	37.11	3.0	1.0	1.0	1.2
C	49.32	3.0	3.0	2.0	0.1
D	49.78	3.0	3.0	1.0	0.1
E	49.75	3.0	1.0	2.0	0.1
F	49.46	3.0	1.0	1.0	0.1

Table 3.4. Aldrich lignin Samples analyzed in this work by NMR

Sample No.	Description	NMR	Scans*
1	Aldrich lignin, dissolved in DMSO-d6, not acetylated	AC300	2181
2	Aldrich lignin, acetylated under condition A	AC300	2081
3	Aldrich lignin, acetylated under condition B	AC300	2253
4	Aldrich lignin, acetylated under condition C	AC300	2158
5	Aldrich lignin, acetylated under condition D	AC300	2554
6	Aldrich lignin, acetylated under condition E	AC300	2090
7	Aldrich lignin, acetylated under condition F	AM400	3189
8	Aldrich lignin, acetylated under condition G	AM400	79
9	Aldrich lignin, acetylated under condition H	AM400	75

* NMR operation condition: RD=12.0 s; PW=8.9 for AM400, PW=7.5 for AC300.

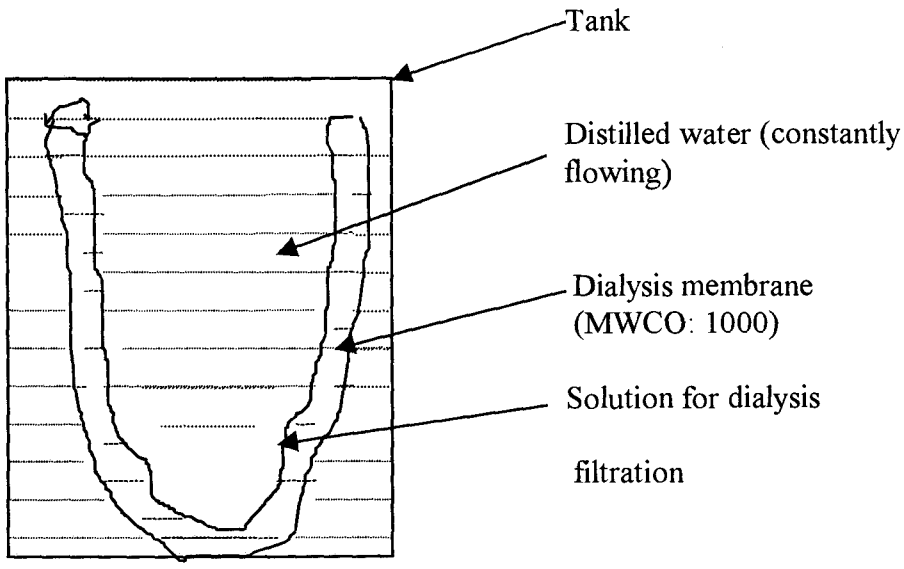

Figure 3.8. Schematic drawing of a simple dialysis filtration

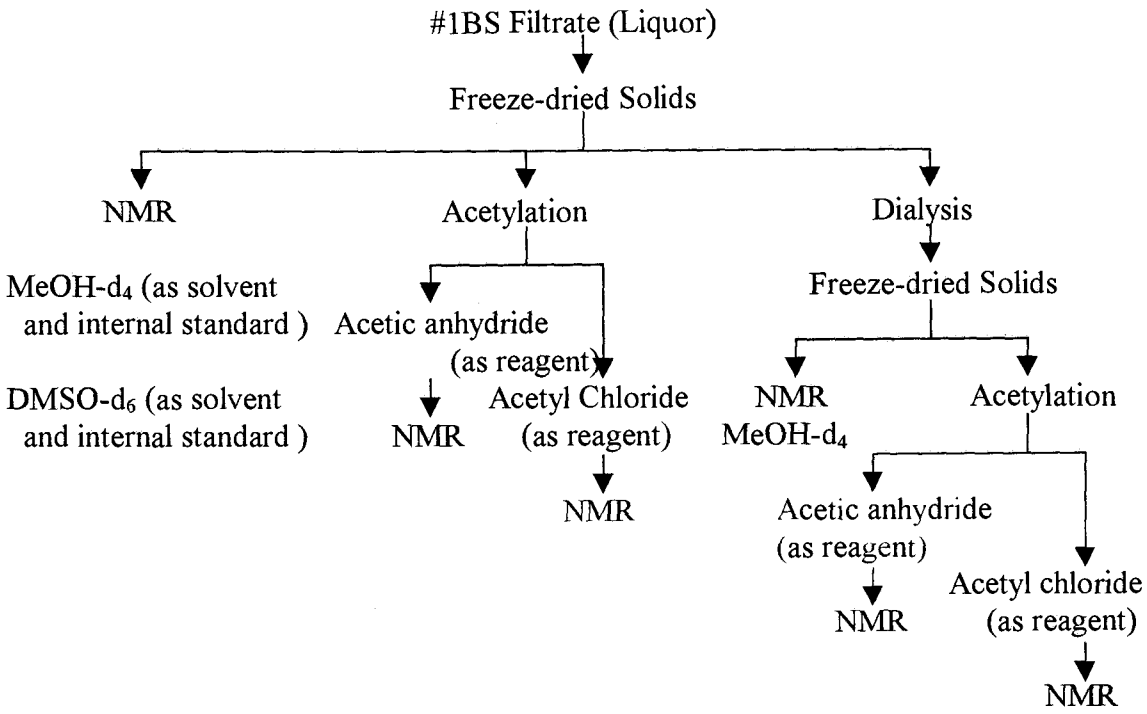

Figure 3.9. Sample preparation for #1BS filtrate before NMR

Table 3.5. Filtrate samples analyzed in this work by NMR

NO.	Description	NMR	Scans
1	#1BS solids (no dialysis), dissolved in DMSO-d6, not acetylated	AC300	17633*
2	#1BS solids (no dialysis), dissolved in MeOH-d4, not acetylated	AC300	18237*
3	#1BS solids (no dialysis), acetylated with acetic anhydride	AM400	4419
4	#1BS solids (no dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	2696
5	#1BS solids (dialysis), dissolved in MeOH-d4, not acetylated	AM400	2550
6	#1BS solids (dialysis), acetylated with acetic anhydride	AM400	2764**
7	#1BS solids (dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	76
8	#2BS solids (dialysis), acetylated with acetic anhydride	AM400	2073
9	#2BS solids (dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	72
10	CB solids (dialysis), acetylated with acetic anhydride	AM400	4558
11	CB solids (dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	315
12	Q solids (no dialysis), acetylated with acetic anhydride	AM400	2125
13	Q solids (dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	566
14	PO2 solids (no dialysis), acetylated with acetic anhydride	AM400	2532
15	PO2 solids (dialysis), acetylated with $CH_3{}^{13}COCl$	AM400	735

* These two NMR conditions: RD=0.0 s, PW=2.3. All others: RD=12.0 s, PW=8.9.

** Both condition of RD=0.5 s and PW=3.0, and condition of RD=12 s and PW=8.9 were tried for this sample

IV. RESULTS AND DISCUSSION

A. Description of Samples

The appearances and pH values of the six filtrates are shown in Table 4.1.

Table 4.1 Appearances and pH values of filtrate samples

No.	Name of sample	Date obtained	Color	Odor	pH Value
1	#1BS Filtrate	08/16/96	Dark Black	Foulest	12.5
2	CB Filtrate	08/16/96	Brown	Fouler	12.0
3	PO_2 Filtrate	08/02/96	Yellow	Foul	11.0
4	Q Filtrate	08/02/96	Light yellow	Little	6.0
5	EOP Filtrate	08/16/96	Little color	Little	10.5
6	P3 Filtrate	08/16/96	Colorless	Odorless	9.5

B. Chemical Contents Analysis

The solids content (SC), suspended solids content (SSC), dissolved solids content (DSC), ash content (AC), moisture content (MC) and Klason lignin content (KLC) were calculated using the following formulas (TAPPI Test Methods 1988).

1. Solids Content (SC):

$$\% \text{ Solids} = \frac{\text{Weight of oven-dried solids}}{\text{Weight of filtrate sample}} \times 100$$

2. Suspended Solids Content (SSC):

$$\% \text{ Suspended Solids} = \frac{\text{Weight of oven-dried suspended solids}}{\text{Weight of filtrate sample}} \times 100$$

3. Dissolved Solids Content (DSC):

$$\% \text{ Dissolved Solids} = \frac{\text{Weight of oven-dried dissolved solids}}{\text{Weight of filtrate sample}} \times 100$$

4. Ash Content (AC):

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Weight of freeze-dried solid filtrate sample}} \times 100$$

5. Moisture Content (MC):

$$\% \text{ Moisture} = \frac{\text{Weight of FDSFS} - \text{Weight of oven-dried FDSFS}}{\text{Weight of freeze-dried solid filtrate sample (FDSFS)}} \times 100$$

6. Klason Lignin Content (KLC):

$$\% \text{ Klason Lignin} = \frac{\text{Weight of Klason Lignin}}{\text{Weight of freeze-dried solid filtrate sample}} \times 100$$

The results of SC, SSC, DSC, AC, MC and KLC of the six filtrates are shown in Table 4.2. Due to lack of samples, we could not determine the Klason lignin contents of PO2, Q, EOP and P3 filtrates after freeze-drying, and the ash content and the moisture content of the PO2 filtrate after freeze-drying.

Solids content (SC) in this experiment means the relative amount of inorganic and organic chemicals in the filtrate. According to TAPPI T650 pm-84, there is a requirement to determine SC, "for weak liquors under 30%, a 5- to 10- g specimen is

Table 4.2 Results of chemical contents in filtrate samples

1	No.	1	2	3	4	5	6
2	Name	#1BS Fil	CB Fil	PO2 Fil	Q Fil	EOP Filt.	P3 Fil
3	SC (%) *	10.94	1.43	0.66	0.39	0.34	0.14
4	SC (%)	10.66	1.32	0.64	0.39	0.36	0.13
5	DSC (%)	11.17	1.41	0.63	0.32	0.31	0.11
6	SSC (%)	0.39	0.050	0.035	0.085	0.035	0.017
7	AC (%)	52.0	65.5	/	72.8	62.0	47.0
8	MC (%)	11.3	7.2	/	2.6	26.6	48.4
9	KLC (%)	25.8	8.2	/	/	/	/

Rows: 1. Sample number
2. Sample identification
3. * Percentage of Solid Contents in the filtrate with 7 g of test specimen
4. Percentage of Solid Contents in the filtrate with 2 g of test specimen
5. Percentage of Suspended Solid Contents in the filtrate
6. Percentage of Dissolved Solid Contents in the filtrate
7. Percentage of Ash Contents in the freeze-dried solid filtrate
8. Percentage of Moisture Contents in the freeze-dried solid filtrate
9. Percentage of Klason lignin in moisture-free freeze-dried solid filtrate

required". To save samples, we tried less amount of sample, 2 g for each filtrate, and compared it with using 7 g for each filtrate. The data in Table 4.2 show that there were no great differences between the results of the solids contents that were obtained with 7 g of specimen and those which were obtained with 2 g of specimen. So it was reliable to measure other contents by using less amount of samples if enough care was taken.

This table shows that for the same amount of filtrate, the #1BS filtrate had the largest solid content (10.94%), and as the pulp was further processed with bleaching stages, the solid contents of the filtrates decreased sharply. This was especially true for the CB filtrate. It decreased to 1.43%, almost 7 times less than that of the #1BS. The P3

filtrate had the lowest amount, 0.13%. This may illustrate why the #1BS filtrate looked darkest and smelled foulest while the P3 filtrate looked and smelled like water.

The suspended solid content also decreases as the pulping and bleaching goes on, although they were very small, less than 0.5%. But the amount will be large enough in a pulp mill because of its high productivity, so that it may be one of the reasons that the pulp mills need to clean up their washers, pipelines and other machines regularly.

The moisture contents show large differences although all the six filtrates were freeze-dried under almost the same conditions. There still was 48.4% moisture in the freeze-dried solids of the P3 filtrate, which is the largest and may indicate there are a lot of hydrophilic chemicals present, such as polysaccharides.

The ash content means the relative amount of inorganic chemical in the filtrate. They show no great differences. However, the calculations were based on the freeze-dried solids of the filtrates, which have different moisture contents. So are the Klason lignin contents. In order to have a much clearer comparison, we obtained a Table 4.3 from the Table 4.2, in which all data were calculated on the basis of 1000 g of filtrate sample.

Table 4.3 shows clearly that there were very little dissolved organic chemicals in most of the filtrate samples (Q, EOP and P3), which indicated it would be very difficult to analyze the dissolved organic chemicals, particularly for the analysis of lignin, which is our main interest. The amounts of ash, lignin and other organic chemicals in the #1BS filtrate were largest, so it was easier to analyze #1BS filtrate.

Figure 4.1 is taken from Table 4.3. It also shows clearly the relationships of the chemical contents in the different filtrates. The ash content decreases sharply from 65.49

g per 1000 g of filtrate to only 1.27 g per 1000 g of filtrate as the pulping and bleaching processes continued. So does the organic content in the filtrate.

Table 4.3 Inorganic and organic chemicals contents in filtrate samples

(per 1000 g of the filtrate sample)

1	No.	1	2	3	4	5	6
2	Name	#1BS Fil	CB Fil	PO2 Fil	Q Fil	EOP Fil	P3 Fil
3	Water (a)	874.06	984.59	/	996.0	995.37	997.29
4	Water (b)	13.85	1.08	/	0.12	1.25	1.30
5	Water (c)	887.91	985.67	/	996.12	996.62	998.59
6	Ash	65.49	10.17	/	2.92	2.87	1.27
7	Organic	46.60	4.16	/	1.00	0.51	0.14
8	Lignin	32.74	1.23	/	/	/	/
9	Others*	13.85	2.93	/	/	/	/

Rows: 1. Sample number
2. Sample identification
3. Weight of moisture in the filtrate sample removed by freeze-drying
4. Weight of moisture in the freeze-dried solid filtrate sample
5. Weight of moisture in the filtrate sample
6. Weight of ash (inorganic chemicals) in the filtrate sample
7. Weight of organic chemicals in the filtrate sample
8. Weight of Klason lignin in the filtrate sample
9. Weight of other organic (including polysaccharides and wood extractives)

As reference, the chemical contents (MC and KLC) of the six pulp samples were determined. Results are shown in Table 4.4. Generally, the moisture content increases as the Klason lignin content decreases because of the presence of less hydrophobic chemicals and more hydrophilic chemicals. Compared with the P3 filtrate, the P3 pulp also has the lowest content of Klason lignin and highest content of water.

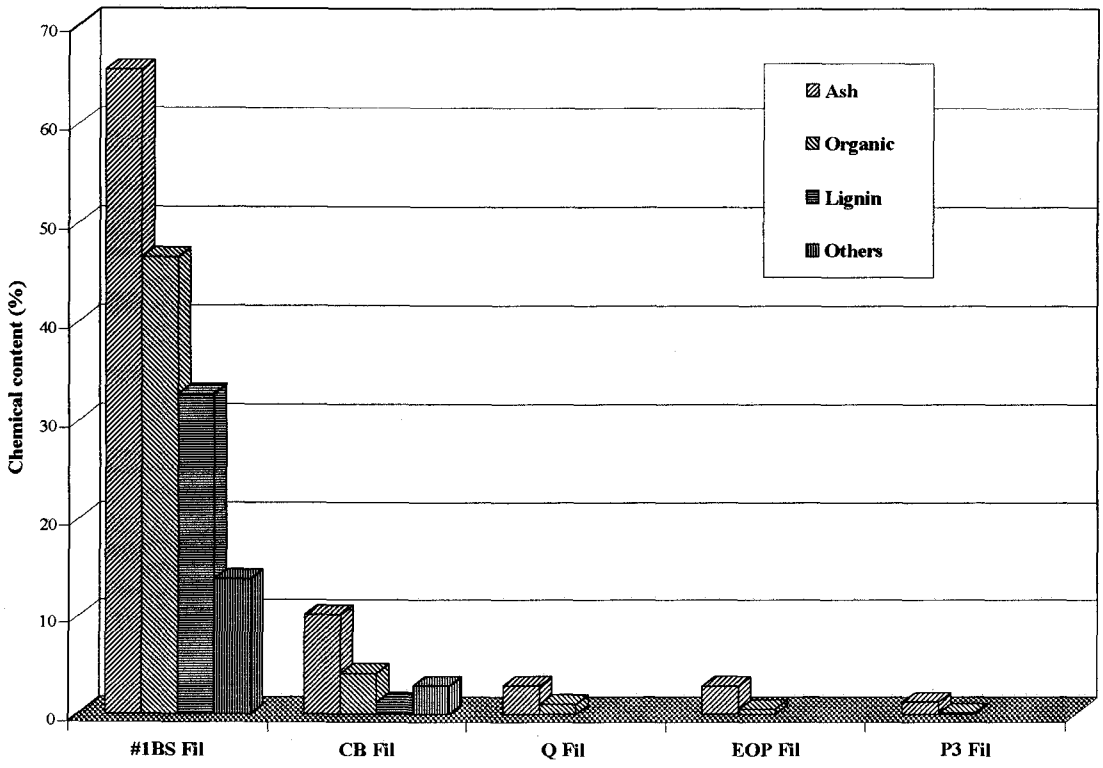

Figure 4.1 Chemical contents of the six filtrate samples

Table 4.4 Chemical contents (MC and KLC) of pulp samples

1	No.	1	2	3	4	5	6
2	Name	#2BS Pulp	Q Pulp	EOP Pulp	P1 Pulp	P2 Pulp	P3 Pulp
3	MC (%)	62.6	77.0	49.5	63.6	82.9	80.3
4	KLC (%)	2.97	1.15	0.72	0.10	0.24	0.23

Rows:

1. Sample number
2. Sample identification
3. Percentage of Moisture Contents in the pulp sample
4. Percentage of Klason Lignin Contents in the pulp sample

C. Thin Layer Chromatography (TLC)

Thin layer chromatography results can be quantitatively analyzed by R_f, R_s (relative resolution), and R_x (relative retention) using following formulas to calculate them (R. J. Hamilton and S. Hamilton, 1987; J. C. Touchstone, 1992):

$$R_f = \frac{X}{Y}$$

X--Distance of compound from origin

Y--Distance of solvent front from origin

$$R_s = \frac{dx}{0.5(d1+d2)}$$

dx--distance between the center of two spots

d1, d2--average diameters of the spots

$$R_x = \frac{X}{Z}$$

X--Distance of compound from origin

Z--Distance of reference compound from origin

C.1 Characterization of Monosaccharides by TLC

The TLC results of the five authentic monosaccharides, D-xylose, L-arabinose, D-mannose, D-glucose and D-galactose, are shown in Table 4.5.

Pentoses run faster than hexoses on the pre-coated cellulose plates, and D-xylose runs faster than L-arabinose. Of the three hexoses, D-mannose runs fastest, D-glucose second, and the slowest is D-galactose. The different absorbing and desorbing effects that are affected by their different chemical structures influence this.

The R_f values are characteristic of the monosaccharides, so TLC can be done on different sizes of pre-coated cellulose plates. D-xylose has the largest R_f value of 0.51, while D-galactose has the smallest R_f value of 0.23. The relative resolution (R_s) values between two close monosaccharides were always larger than 1.0, which means all

Table 4.5. Results of authentic sugars after three-time development in TLC

[Developing solvent (acetate-pyridine-distilled Water 8:2:1 v/v)]

1	Plate No.	1	2	3	4	5	Average
2	Y (cm)	15.70	16.20	15.90	17.00	17.10	16.38
3	X, xylose (cm)	7.00	9.67	9.35	8.10	8.00	8.42
4	X, arabinose(cm)	5.50	8.10	8.00	6.60	6.45	6.93
5	X, mannose(cm)	4.40	7.30	7.20	5.65	5.35	5.98
6	X, glucose(cm)	3.30	5.90	5.95	4.20	4.00	4.67
7	X, galactose(cm)	2.66	4.75	4.80	3.30	3.05	3.71
8	d, xylose(cm)	0.90	0.85	0.85	0.90	0.90	0.88
9	d, arabinose(cm)	0.80	0.85	0.85	0.90	0.90	0.86
10	d, mannose(cm)	0.80	0.85	0.80	0.85	0.85	0.83
11	d, glucose(cm)	0.75	0.90	0.80	0.80	0.85	0.82
12	d, galactose(cm)	0.80	0.80	0.80	0.80	0.80	0.80
13	R_f, xylose	0.45	0.60	0.59	0.48	0.47	0.51
14	R_f, arabinose	0.35	0.50	0.50	0.39	0.38	0.42
15	R_f, mannose	0.28	0.45	0.45	0.33	0.31	0.36
16	R_f, glucose	0.21	0.36	0.37	0.25	0.23	0.28
17	R_f, galactose	0.17	0.29	0.30	0.19	0.18	0.23
18	R_s, xyl-arabinose	1.76	1.85	1.59	1.67	1.72	1.72
19	R_s, ara-mannose	1.38	0.94	0.97	1.09	1.28	1.13
20	R_s, man-glucose	1.33	1.60	1.56	1.78	1.59	1.57
21	R_s, glu-galactose	0.78	1.40	1.44	1.13	1.15	1.18
22	R_x, xyl-galactose	2.62	2.04	1.95	2.45	2.62	2.34
23	R_x, ara-galactose	2.07	1.70	1.67	2.00	2.11	1.91
24	R_x, man-galactose	1.65	1.54	1.50	1.71	1.75	1.63
25	R_x, glu-galactose	1.24	1.24	1.24	1.27	1.31	1.26

Rows: 1. TLC plate number that was used at different time
2. The distance of solvent front from origin
3-7. The distance of different authentic monosaccharide from origin
8-12. The diameter of different authentic monosaccharide
13-17. The R_f value of different authentic monosaccharide
18-21. The relative resolution (R_s) between two close authentic monosaccharide
22-25. The relative retention (R_x) of the different authentic monosaccharide referred to the galactose

the monosaccharides were separated well enough. The relative retention (R_x) values also showed this point.

These results in Table 4.5 were also illustrated by Figure 4.2.

The developing solvent (ethyl acetate-pyridine-distilled water, 8:2:1 v/v) was a good solvent system to identify the monosaccharides by TLC.

No simple free monosaccharides existed in any of the six filtrate samples as detected by TLC using this developing solvent system (Table 4.6). Neutralization and concentration of the filtrate samples still did not show any monosaccharides. However, after hydrolysis by 72.0% or by 77.0% sulfuric acid, all five types of monosaccharides were found in the #1BS, CB, PO2, Q and EOP filtrate, but none were found in the P3 filtrate.

Thus, we can conclude that there are only polysaccharides but no free monosaccharides in the #1BS, CB, PQ2, Q and EOP filtrate, and that neither polysaccharides nor monosaccharides were in the P3 filtrate. These polysaccharides contained both cellulose and hemicelluloses.

C.2 Characterization of Wood Extractives by TLC

Compared to the wood extractives in the filtrate samples, the pure authentic reference compounds, quercetin (Q), dihydroquercetin (DHQ) and (+)-catechin (C), were better separated and identified by TLC because there was no interference from polysaccharides and lignin as with the filtrate samples.

In this experiment, we found that pre-coated silica plates were better than pre-coated cellulose plates to separate the three extractives even when the two dimensional

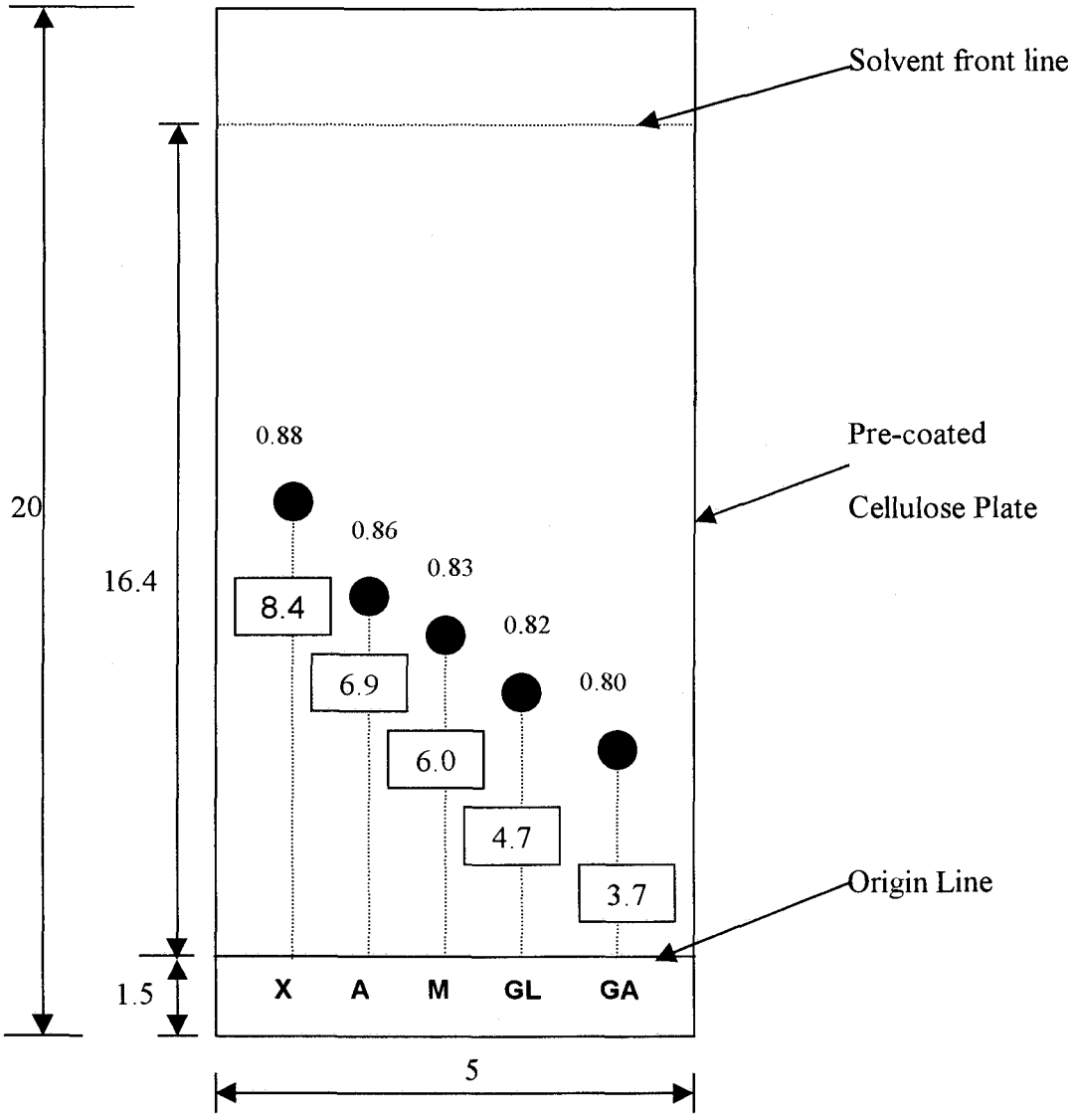

Figure 4.2 Results of authentic sugars after three-time development in TLC

(X: D-xylose; A: L-arabinose; M: D-mannose; GL: D-glucose; GA: D-galactose)

(Unit: cm)

Table 4.6 Characterization of monosaccharides in filtrates by TLC

N0.	sample name	Monosaccharides in filtrate sample	Monosaccharides in hydrolysate	Polysaccharides in filtrate sample
1	#1BS Filtrate	No	Yes	Yes
2	CB Filtrate	No	Yes	Yes
3	PO_2 Filtrate	No	Yes	Yes
4	Q Filtrate	No	Yes	Yes
5	EOP Filtrate	No	Yes	Yes
6	P3 Filtrate	No	No	No

(2-D) TLC method was applied. The three-time developing method was needed to have good resolution. The optimum developing solvent systems to separate the authentic extractives were: Benzene-Acetone-Methanol (6:3:1), Chloroform-EtOAc-Formic acid (3:1:1), and Toluene-Acetone-Formic acid (7:3:1) and (5:3:1). The other systems did not separate the authentic extractives well enough. For example, when using Chloroform-EtOAc-Methanol (3:1:2), (2:1:3), (3:1:1) or (2:1:2) as developing solvent, the samples all either moved too fast [(2:1:3), (2:1:2)] or hardly moved [(3:1:2), (3:1:1)] so that no separation occurred.

The results of the three authentic wood extractives after three times of developing in the solvent systems, Toluene-Acetone-Formic acid (5:3:1) (solvent A) and (7:3:1) (solvent B), and Chloroform-EtOAc-Formic acid (3:1:1) (solvent C), are shown in Table 4.7, Table 4.8, and Table 4.9 respectively. These three tables show that quercetin (Q) runs faster than dihydroquercetin (DHQ), and that both Q and DHQ run faster than C.

According to the R_f, Rs and Rx values, all of these three developing solvent systems were applicable to separate the authentic wood extractives effectively.

Table 4.7 Results of authentic extractives developed in Solvent A in TLC

[Solvent A: toluene-acetone-formic Acid (5:3:1)]

1	Plate No.	1	2	3	4	Average
2	Y (cm)	7.52	7.48	7.40	7.58	7.50
3	X, quercetin (cm)	5.10	5.15	4.95	5.25	5.11
4	X, dihydroquercetin (cm)	4.55	4.60	4.40	4.50	4.51
5	X, catechin (cm)	4.00	4.00	4.00	4.05	4.01
6	D, quercetin (cm)	0.20	0.20	0.22	0.25	0.22
7	D, dihydroquercetin (cm)	0.30	0.30	0.30	0.25	0.29
8	D, catechin (cm)	0.20	0.20	0.30	0.30	0.25
9	Rf, quercetin	0.69	0.69	0.67	0.69	0.69
10	Rf, dihydroquercetin	0.61	0.61	0.59	0.59	0.60
11	Rf, catechin	0.53	0.53	0.54	0.53	0.53
12	Rs, quer-dihydro	2.20	2.20	2.50	3.00	2.48
13	Rs, dihydro-catechin	2.20	2.40	1.60	1.80	2.00
14	Rx, quer-catechin	1.28	1.29	1.24	1.30	1.27
15	Rx, dihydro-catechin	1.14	1.15	1.10	1.11	1.12

Rows:

1: TLC plate number that the plate was runned at different time
2: The distance of solvent front from origin line in plate
3-5: The distance of different authentic extractive (Quercetin, Dihydroquercetin and Catechin) migrated from the origin line
6-8: The average diameter of different authentic extractives
9-11: The Rf value of different authentic extractives
12-13: The relative resolution between two close extractives
14-15: The relative retention value between two extractives

Table 4.8 Results of authentic extractives developed in Solvent B in TLC

[Solvent B: toluene-acetone-formic Acid (7:3:1)]

1	Plate No.	1	2	3	4	Average
2	Y (cm)	7.85	7.70	7.60	8.00	7.79
3	X, quercetin (cm)	4.00	3.95	3.75	3.75	3.86
4	X, dihydroquercetin (cm)	3.30	3.25	3.05	2.95	3.14
5	X, catechin (cm)	2.40	2.30	2.50	2.40	2.40
6	d, quercetin (cm)	0.20	0.20	0.20	0.25	0.21
7	d, dihydroquercetin (cm)	0.30	0.30	0.25	0.30	0.29
8	d, catechin (cm)	0.35	0.35	0.30	0.25	0.31
9	Rf, quercetin	0.51	0.51	0.49	0.47	0.50
10	Rf, dihydroquercetin	0.42	0.42	0.40	0.37	0.40
11	Rf, catechin	0.31	0.30	0.33	0.30	0.31
12	Rs, quer-dihydro	2.80	2.80	2.70	2.90	2.80
13	Rs, dihydro-catechin	2.80	2.90	2.00	2.00	2.43
14	Rx, quer-catechin	1.67	1.72	1.50	1.56	1.61
15	Rx, dihydro-catechin	1.38	1.41	1.22	1.23	1.31

Rows:

1: TLC plate number that the plate was used at different time
2: The distance of solvent front from origin line in plate
3-5: The distance of different authentic extractive (Quercetin, Dihydroquercetin and Catechin) migrated from the origin line
6-8: The average diameter of different authentic extractives
9-11: The Rf value of different authentic extractives
12-13: The relative resolution between two close extractives
14-15: The relative retention value between two extractives

Table 4.9 Results of authentic extractives developed in Solvent C in TLC

[Solvent C: chloroform-ethyl acetate-formic Acid (3:1:1)]

1	Plate No.	1	2	3	4	Average
2	Y (cm)	7.35	7.60	7.70	6.70	7.34
3	X, quercetin (cm)	5.40	5.50	5.80	5.00	5.43
4	X, dihydroquercetin (cm)	3.60	3.75	4.20	3.55	3.78
5	X, catechin (cm)	2.00	2.05	2.15	2.10	2.08
6	d, quercetin (cm)	0.30	0.35	0.30	0.30	0.31
7	d, dihydroquercetin (cm)	0.60	0.65	0.55	0.50	0.58
8	d, catechin (cm)	0.20	0.20	0.50	0.30	0.30
9	Rf, quercetin	0.73	0.72	0.75	0.75	0.74
10	Rf, dihydroquercetin	0.49	0.49	0.55	0.52	0.51
11	Rf, catechin	0.27	0.27	0.28	0.31	0.28
12	Rs, quer-dihydro	4.00	3.50	3.80	3.60	3.73
13	Rs, dihydro-catechin	4.00	4.00	3.90	3.60	3.88
14	Rx, quer-catechin	2.70	2.68	2.70	2.38	2.62
15	Rx, dihydro-catechin	1.80	1.83	1.95	1.69	1.82

Rows:

1: TLC plate number that the plate was used at different time
2: The distance of solvent front from origin line in plate
3-5: The distance of different authentic extractive (Quercetin, Dihydroquercetin and Catechin) migrated from the origin line
6-8: The average diameter of different authentic extractives
9-11: The Rf value of different authentic extractives
12-13: The relative resolution between two close extractives
14-15: The relative retention value between two extractives

Interestingly, we found that the formic acid played a very important role in separating these extractives. If there was no formic acid in a solvent system, the relative resolution would be very poor although the total polarity of the solvent system was very high. For example, Chloroform-EtOAc-Methanol (6:1:3) was not useful because of no reasonable resolution obtained during separation. The reason is still not understood.

The solvent system, Benzene-Acetone-Methanol (6:3:1), was not used because when we applied this system to the pulp filtrate samples, it did not separate the wood extractives well enough. However, the developing solvents A, B and C were effective.

Table 4.10 shows the results of TLC when different procedures of sample preparation using different solvents to extract were performed, no matter what kind of developing system was used. There were no extractives in the ethyl acetate solution that could be detected by TLC. This could be due to the low polarity of this solvent.

Three kinds of extractives all showed in the #1BS and CB filtrates by TLC (Figure 4.3), but they had different contents in the filtrate samples according to the TLC plates. Firstly, we did not concentrate the #1BS extractives as much as we did to the CB extractives, so there should be more contents of wood extractives in the #1BS filtrate.

Table 4.10 Characterization of wood extractives in filtrates by TLC

N0.	Sample Name	Methanolic Extractives	Water Extractives	Ethyl Acetate Extractives
1	#1BS Filtrate	Yes	Yes	No
2	CB Filtrate	Yes	Yes	No
3	PO_2 Filtrate	No	No	No
4	Q Filtrate	No	No	No
5	EOP Filtrate	No	No	No
6	P3 Filtrate	No	No	No

Secondly, catechin often showed up in TLC plates while DHQ and Q only showed up sometimes, so there should be more contents of catechin existing in #1BS and CB filtrates. Considering the fact that dihydroquercetin has proven to be the main extractive in Douglas-fir [Laver and Wang 1996], we suggested that during pulping and bleaching processes, most of the dihydroquercetin might have been chemically changed into catechin and other chemicals. Also, as in Figure 4.3, there were some other chemicals existing in the #1BS and CB filtrates.

Table 4.11 shows the results of different solubilities of the #1BS filtrate freeze-dried solids dissolved in these three extractive solvents. #1BS freeze-dried solids can be almost completely dissolved in distilled water, but only about half can be dissolved in methanol, and hardly any dissolved in ethyl acetate. This may explain why no extractives in the EtOAc solution were detected by TLC.

Table 4.11 Solubility of #1BS freeze-dried solids in different solvents

Name of Extractive	MeOH	EtOAc	H_2O
Solubility of #1BS Solids	66.1%	11.2%	99.6%

D. Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR)

NMR spectra were obtained for both Aldrich lignin and filtrates lignin under variable conditions. Most of them are attached in the APPENDIX part of this thesis.

The results show that the NMR spectra of lignin are separated into four functional group regions: carbonyl carbons (between 165 ppm and 190 ppm), aromatic carbons (between 104 ppm and 164 ppm), carbons single-bonded to oxygen (C-O,

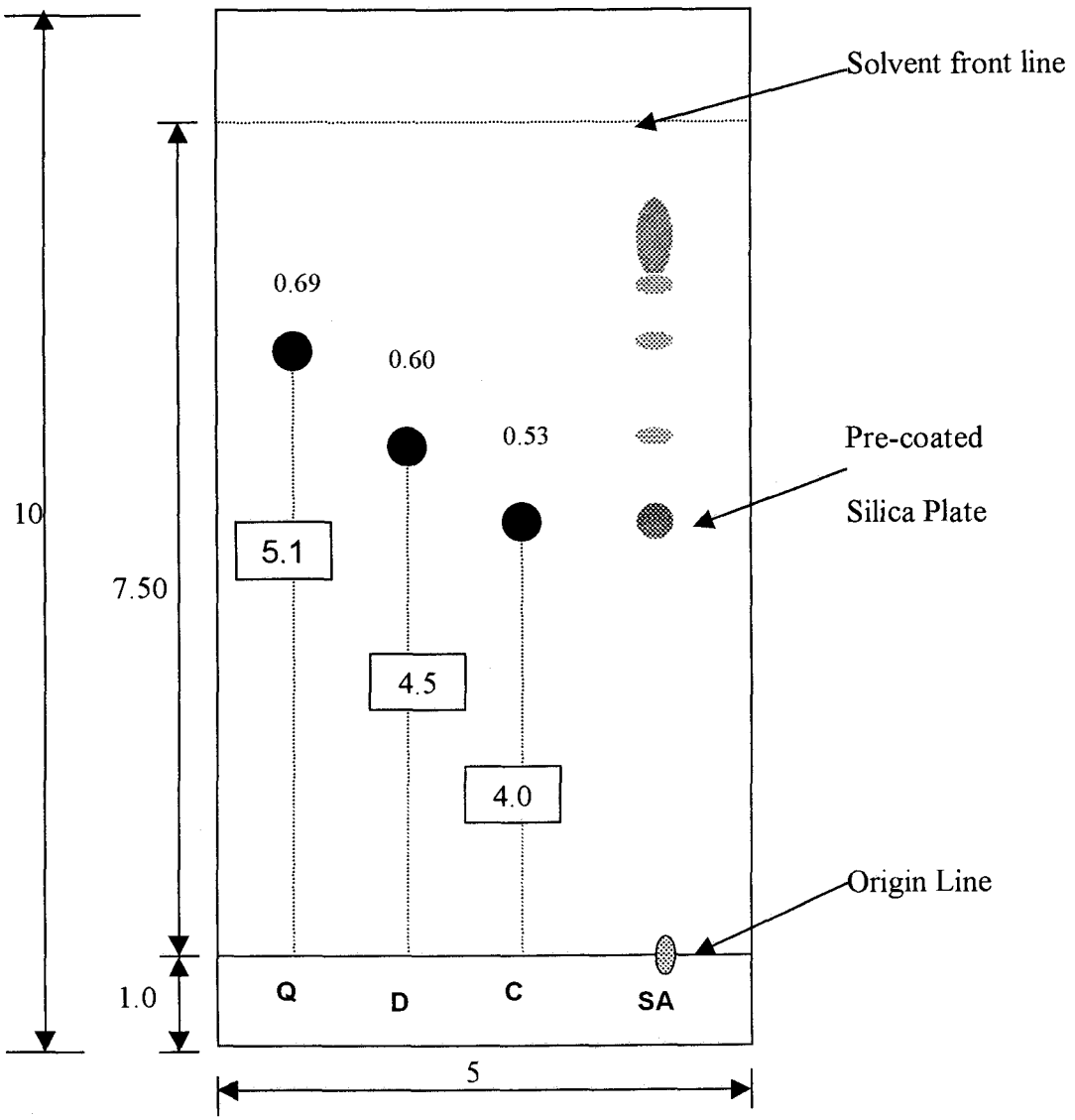

Figure 4.3. Results of authentic extractives and filtrate sample after three-time development in TLC. [Solvent A: toluene-acetone-formic Acid (5:3:1)]

(Q-quercetin; D-dihydroquercetin; C-catechin; SA-sample)

between 60 ppm and 84 ppm), and aliphatic carbons (between 12 ppm and 36 ppm). The peak at 55.6 ppm is the characteristic methoxyl group in the guaiacyl units of lignin (Robert 1992; Wilson 1987).

D.1 Characterization of Aldrich Lignin by ^{13}C-NMR

Figure 4.4 shows the spectrum of Aldrich lignin dissolved in DMSO-d_6 without acetylation. When preparing this sample, the purchased Aldrich lignin was found not to completely dissolve in the DMSO-d_6 solvent.

No carbonyl carbons or aliphatic carbons were detected in this figure. The aromatic carbons and methoxyl groups were shown well. If given more time to run NMR, the resolution would be good enough for quantification. This may be one good way to differ softwood lignin from hardwood lignin because they have different methoxyl contents per aryl group.

Figure 4.5 shows the spectrum of the Aldrich lignin after acetylation with acetic anhydride under the optimum acetylation condition (Condition E). The other spectra under condition A, B, C, D and F can be seen in the APPENDICES part. Compared with these spectra, the spectrum under Condition E looks better from its signal-to-noise ratio and peaks identification.

The most important difference between Figure 4.4 and Figure 4.5 is that Figure 4.5 gives more information about the Aldrich lignin. Two more main functional group peaks were shown in Figure 4.5: the hydroxyl groups, which clearly include primary hydroxyl group at 170.0 ppm, secondary hydroxyl groups at 169.2 ppm and phenolic hydroxyl groups at 168.5 ppm, and the methyl group (-CH3) at 20.4 ppm.

In Figure 4.5, there appears to be a little more noise than in Figure 4.4. This noise may be caused by the addition of the acetylation reagent and the catalyst. More running time for NMR should be required if more accurate quantification is needed.

Table 4.12 shows the yields of lignin acetates under different acetylation conditions. The largest yields were 106.3% under Condition E with acetic anhydride, and 103.8% under Condition G with acetyl chloride. These also demonstrated that the Condition E and Condition G were the optimum conditions.

Figure 4.6 shows the spectra of the Aldrich lignin after acetylation with acetyl chloride under different acetylation conditions (Condition G and H) or different NMR operation conditions (different scan number, i.e, different data acquisition time of NMR). There is much more noise in the spectrum of "Condition H", which indicates that the process of acetylation should be first adding acetyl chloride then DMAP (Condition G).

Table 4.12 Yields of Aldrich lignin acetates under different acetylation conditions

Condition	Aldrich Lignin (mg)	Lignin Acetates (mg)	Yield *
Condition C	49.32	29.44	59.7%
Condition D	49.78	43.00	86.4%
Condition E	49.75	52.88	106.3%
Condition F	49.46	43	86.9%
Condition G	52.76	54.76	103.8%
Condition H	54.40	27.52	50.6%

Notes: 1. * Yield = Weight of Product / Weight of Raw Materials x 100%
2. No measurements conducted for Condition A and B.

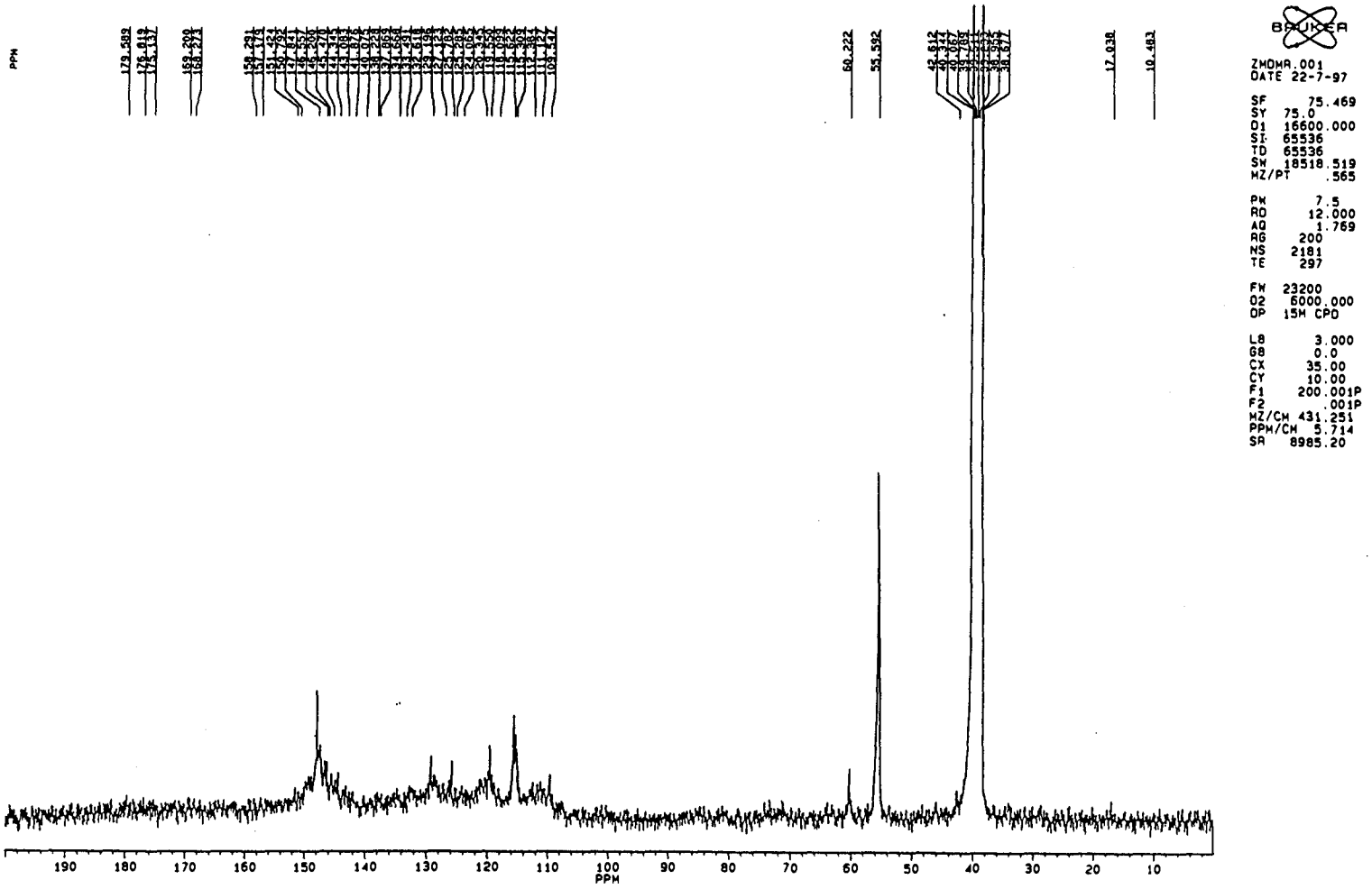

Figure 4.4. ^{13}C-NMR spectrum of Aldrich lignin dissolved in DMSO-d_6 without acetylation (DMSO-d_6 also as internal standard. RD=12 seconds, PW=7.5, NS=2181)

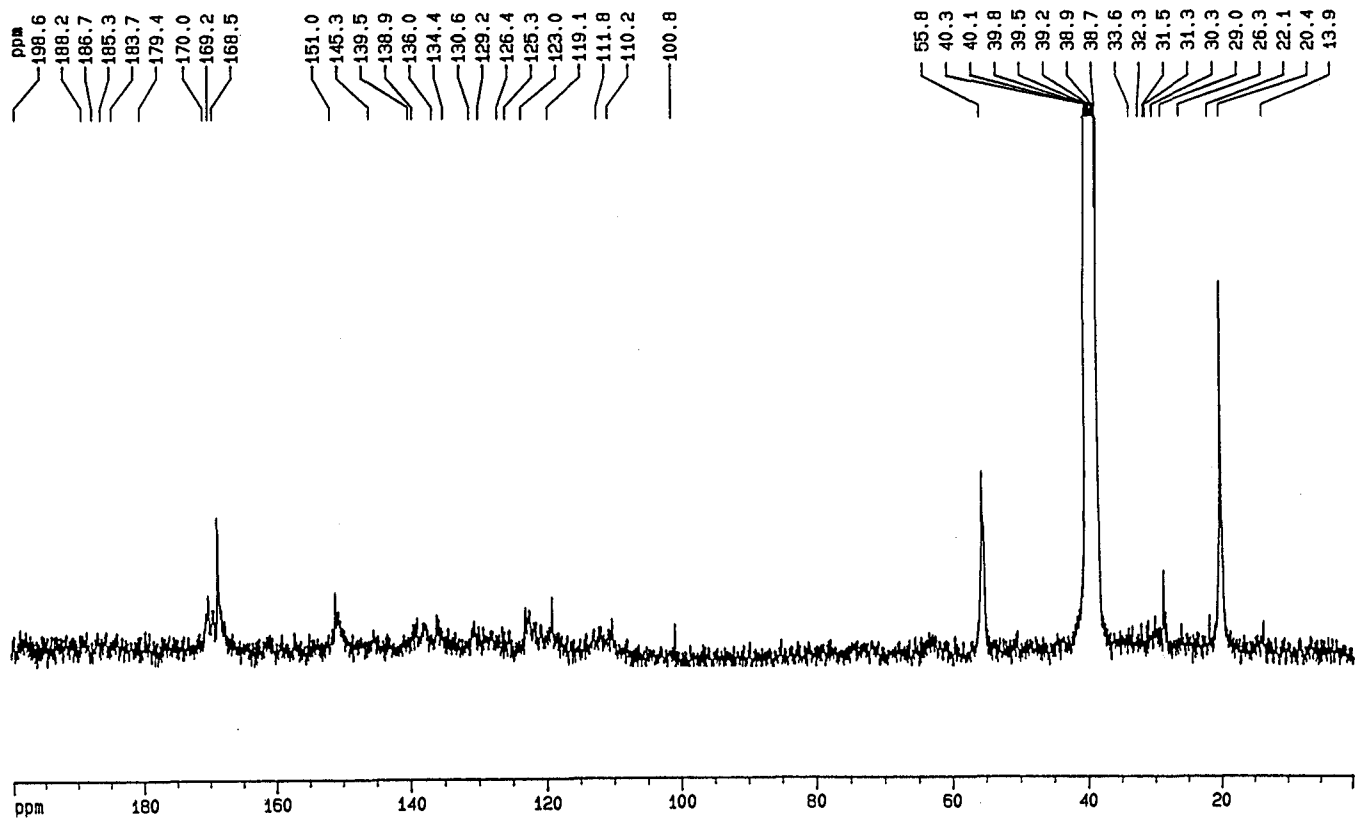

Figure 4.5. ^{13}C-NMR spectrum of Aldrich lignin acetylated under Condition E.
(DMSO- d_6 as solvent and internal standard. RD=12 seconds, PW=7.5, NS=2090)

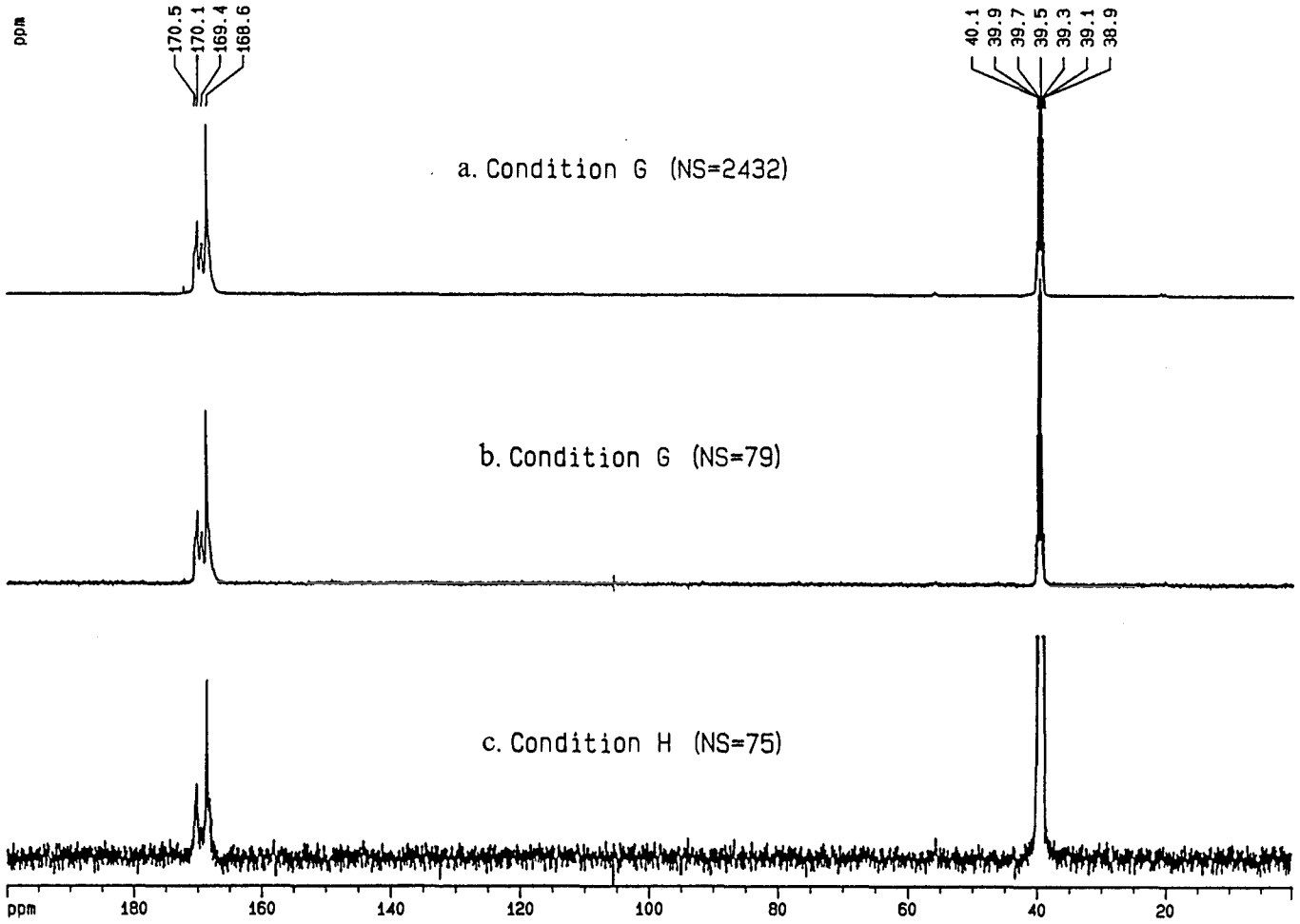

Figure 4.6. ^{13}C-NMR spectra of Aldrich lignin acetylated by acetyl chloride.
a. Condition G (NS=2432); b. Condition G (NS=79); c. Condition H (NS=75).

The spectrum of the larger scan number (NS=2432) shows little difference from that of the small scan number (NS=79). So for the purpose of saving NMR running time, 80 of scan number will be enough to get information for the purified commercial lignin.

The other important result from Figure 4.6 is that after acetylation with acetyl chloride, the three hydroxyl groups can be more accurately quantified by NMR because of its much better signal-to-noise ratio (S/N). But using this acetylation method, there are no other functional groups showing in the spectra, which appear in Figure 4.5.

Figure 4.7 is one part of Figure 4.6 ranged from 180 ppm to 160 ppm. It strongly supports the above discussion in a much cleaner way.

So acetylation with acetyl chloride is a more useful way to quantitatively determine hydroxyl groups in lignin because of its higher resolution and higher speed, but it is not applicable to quantify other functional groups in lignin compared to the way of acetylation with acetic anhydride.

The amounts of each functional group, expressed as a percent of the total number of carbons, are presented in Table 4.13 while their amounts, expressed as a ratio to the number of aromatic rings (aryl units) in the sample, were presented in Table 4.14. The peak area ratios for the three hydroxyl groups were presented in Table 4.15. Discussions of these data are detailed in a later part of this chapter.

D.2 Characterization of Filtrate Lignin by ^{13}C-NMR

No reasonable spectrum was obtained for #1BS freeze-dried solids without acetylation when using DMSO-d_6 as solvent and internal standard. This may be due to the low solubility of #1BS freeze-dried solids in the DMSO-d_6 solvent.

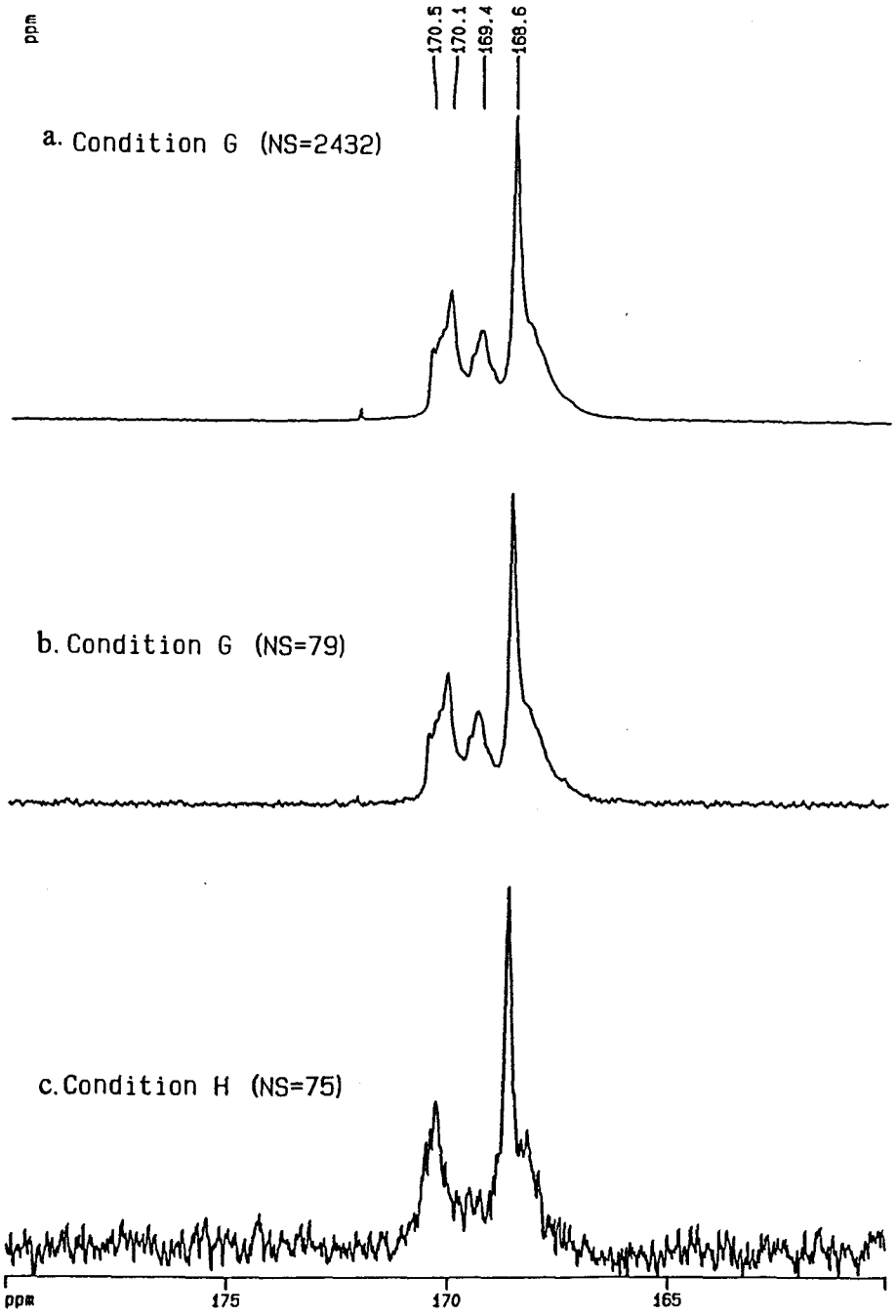

Figure 4.7. Detailed ^{13}C-NMR spectra of Aldrich lignin acetylated by acetyl chloride.

a. Condition G (NS=2432); b. Condition G (NS=79); c. Condition H (NS=75).

Figure 4. 8 shows the spectrum of #1BS freeze-dried solids without acetylation and dialysis when using MeOH-d_4 as solvent and internal standard. A large scan number, 18237, was used. Due to the different solvent, there is some chemical shift difference for same peaks, about 1 ppm. For example, the methyl group, methoxyl group and primary hydroxyl group in Figure 4.8 are at 21.5, 56.5 and 171.1 ppm respectively, but in Figure 4.5 they are at 20.4, 55.6 and 170.0 ppm respectively. From this figure, we know that the lignin in the #1BS filtrate is different from the Aldrich lignin. The lignin of #1BS has a large number of C-O groups (60-84 ppm) and carbonyl groups (180-184 ppm), which the Aldrich lignin does not have.

Compared with Figure 4.8, no reasonable spectrum was obtained for the #1BS freeze-dried solids without acetylation but after dialysis when using MeOH-d_4 as solvent and internal standard. This may be due to less time of NMR running.

Figure 4.9 shows the spectra of the #1BS lignin after acetylation with acetic anhydride and with acetyl chloride, both of which were not dialysis-filtered. Figure 4.10 shows the spectra of the #1BS lignin after acetylation with acetic anhydride and with acetyl chloride, both of which were after dialysis filtration.

All of these four spectra were obtained under almost the same NMR operating conditions except for different scan numbers. Comparing these four spectra, we can get some useful information. First of all, dialysis filtration is important and necessary to get a good NMR spectrum with good resolution and high speed. Secondly, the same as for the Aldrich lignin, it demonstrated that acetylation with acetyl chloride was a more useful way to quantitatively determine hydroxyl groups in #1BS lignin, but the method of acetylation with acetic anhydride gave more peak information about the #1BS lignin

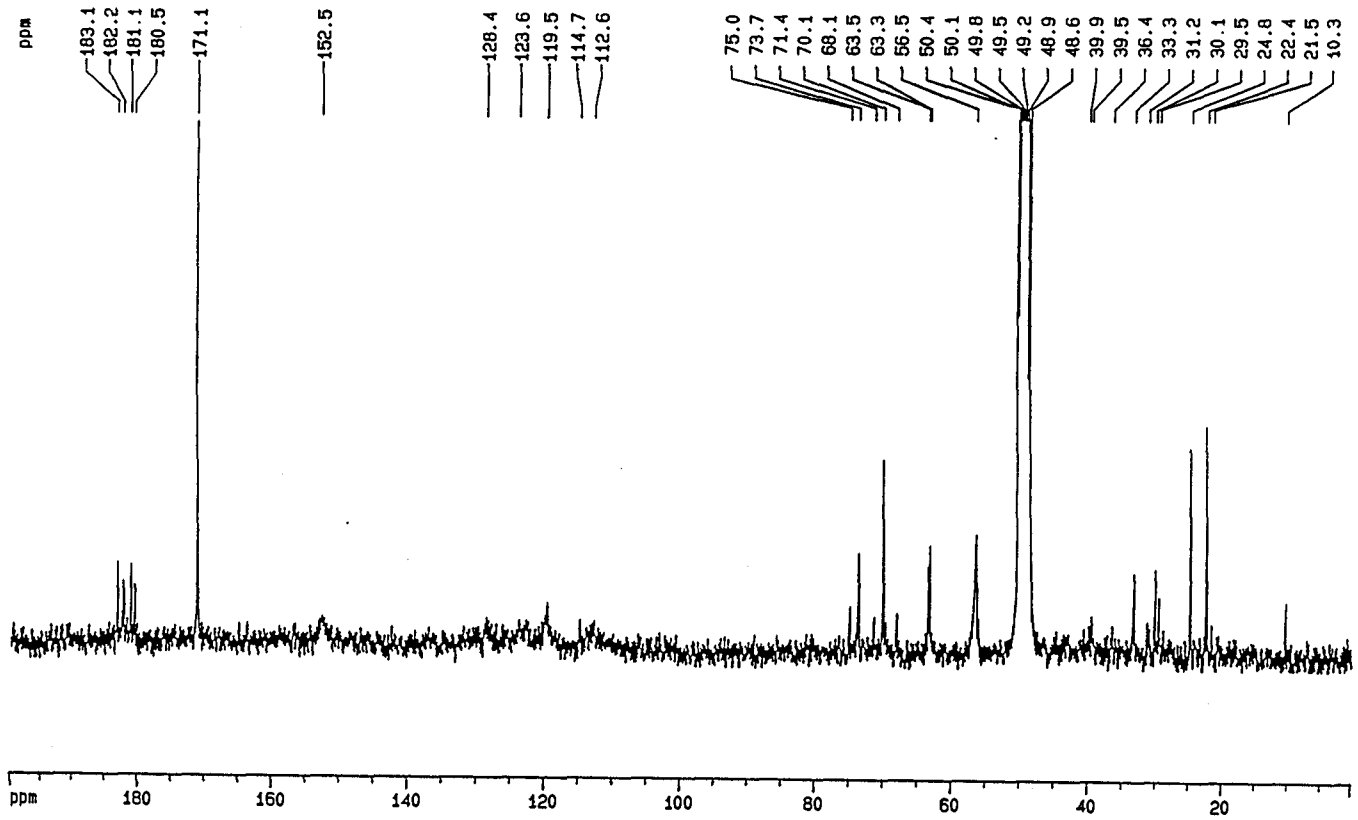

Figure 4.8. ^{13}C-NMR spectrum of #1BS dissolved in MeOH-d_4 without dialysis and acetylation. (MeOH-d_4 also as internal standard. RD=0.5 s, PW=3.0, NS=18237)

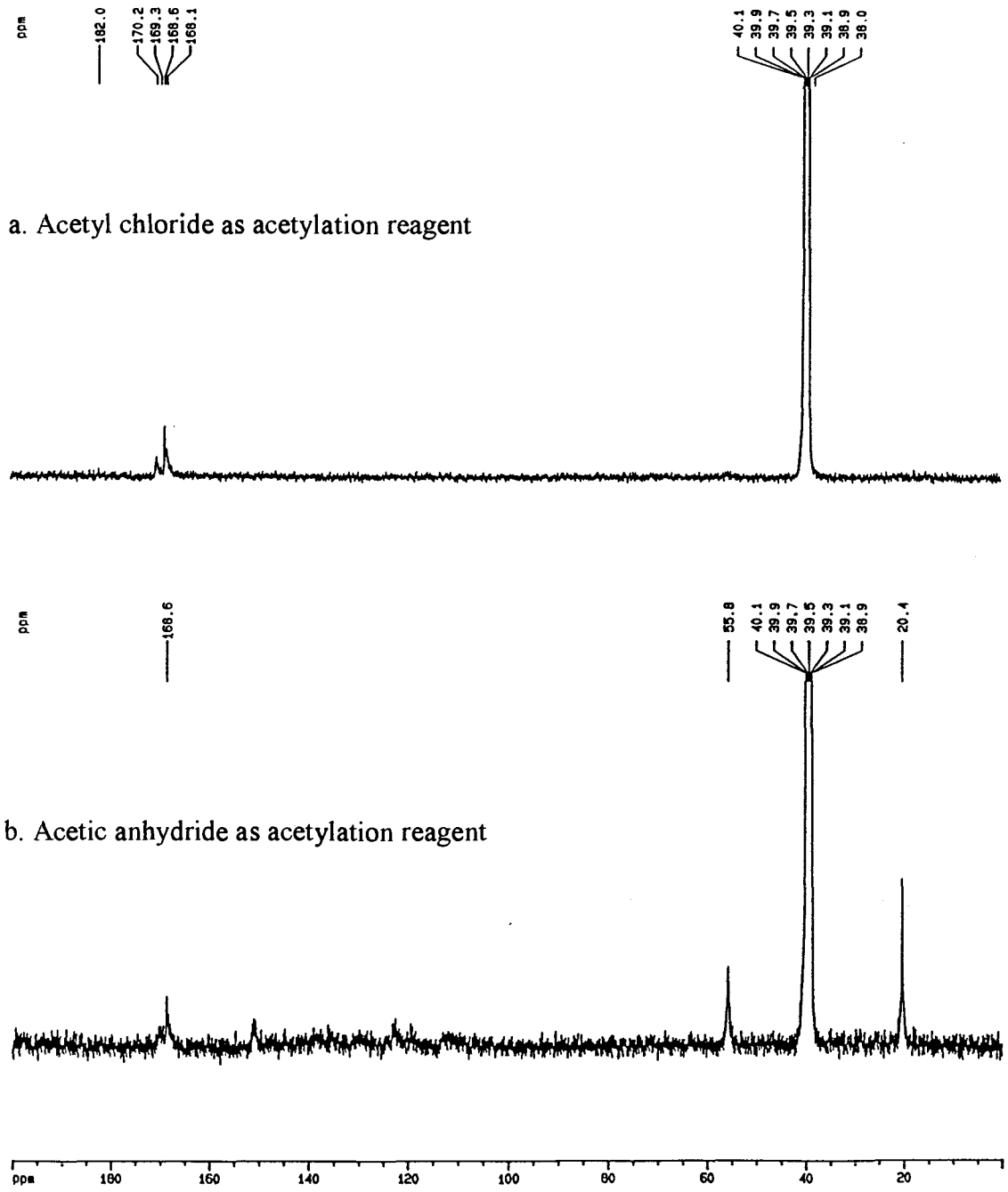

Figure 4.9. ^{13}C-NMR spectra of #1BS acetylated without dialysis filtration before.

a. Acetyl chloride as acetylation reagent; b. Acetic anhydride as acetylation reagent.

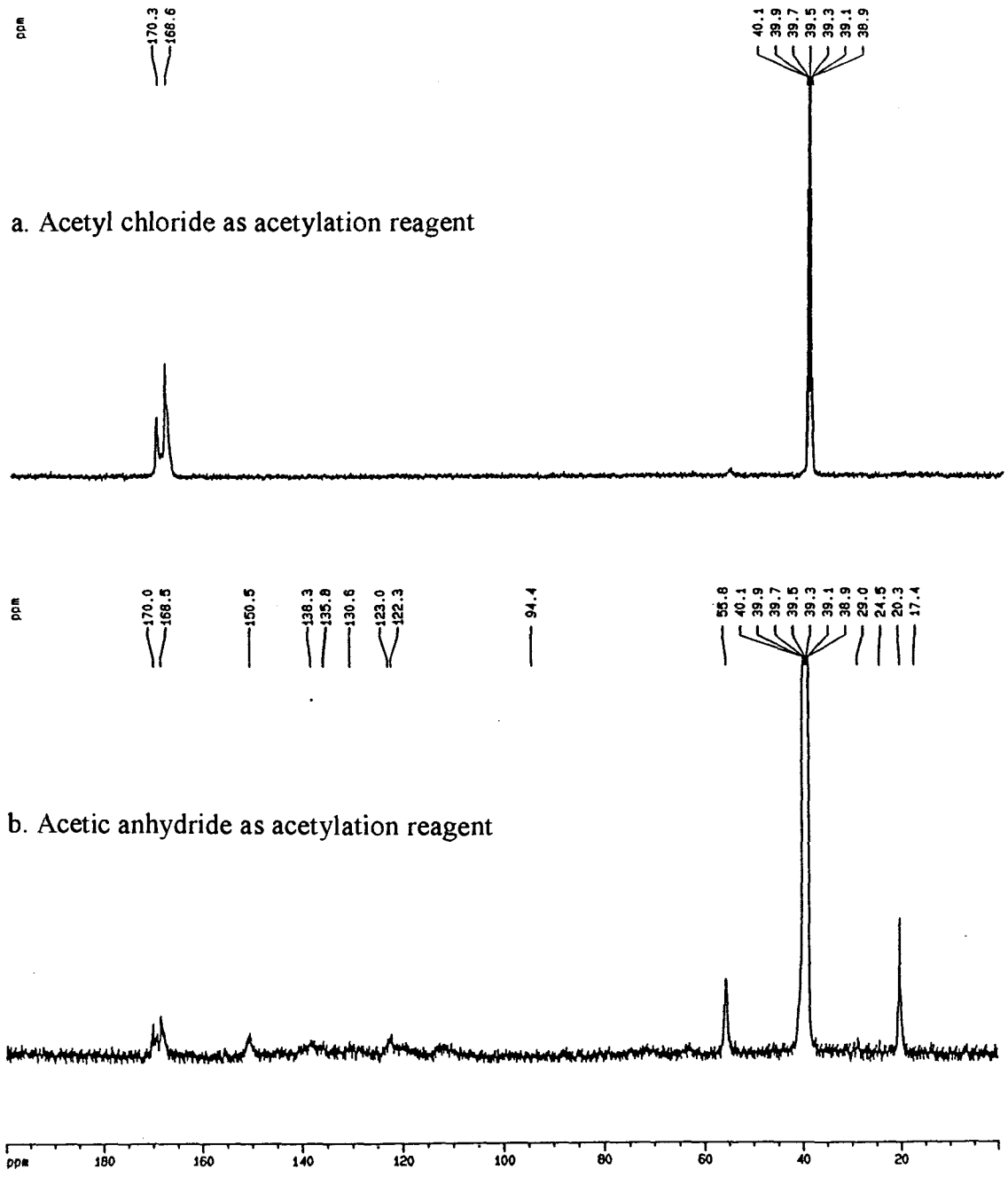

Figure 4.10. ^{13}C-NMR spectra of #1BS acetylated after dialysis filtration.

a. Acetyl chloride as acetylation reagent; b. Acetic anhydride as acetylation reagent.

in NMR spectra. Finally and most importantly, the #1BS lignin was shown to be different from the ordinary softwood kraft lignin (Aldrich lignin) from the amount of hydroxyl groups and other functional groups (see Table 4.13, Table 4.14 and Table 4.14). Discussions are given in a later part.

The changing of NMR conditions from 12 s of RD and 8.9 of PW to 0.5 s of RD and 3.0 of PW did not improve the resolution of the NMR results (see attached Figure A.6 in the part of APPENDIX).

Figure 4.11 shows the spectra of #2BS lignin after dialysis and acetylation with acetyl chloride or with acetic anhydride. The differences between #1BS lignin and #2BS lignin were little, which was confirmed by the fact that no chemicals were added in the pulp mill between these two stages except water.

Figure 4.12 shows the spectra of the CB filtrate lignin after dialysis and acetylation with acetyl chloride. Unfortunately, no useful resolution was obtained. More time of running NMR would be required. However, there is only the primary hydroxyl groups peak identified by NMR, which was very different from #1BS and #2BS lignin. This showed the effect of oxygen delignification on lignin's chemical structure. The disappearance of secondary OH's and phenolic OH's could be due to the fact that they are more active and easier to react than primary OH's.

Figure 4.13 summarizes the spectra of #1BS lignin, #2BS lignin and CB lignin which were acetylated by acetyl chloride after dialysis filtration.

Table 4.13, Table 4.14 and Table 4.15 summarizes the quantitative NMR data for the lignins which were investigated in this research. Because of noise effect, the quantitative values in Table 4.13 and Table 4.14 for the lignin acetic anhydride acetates

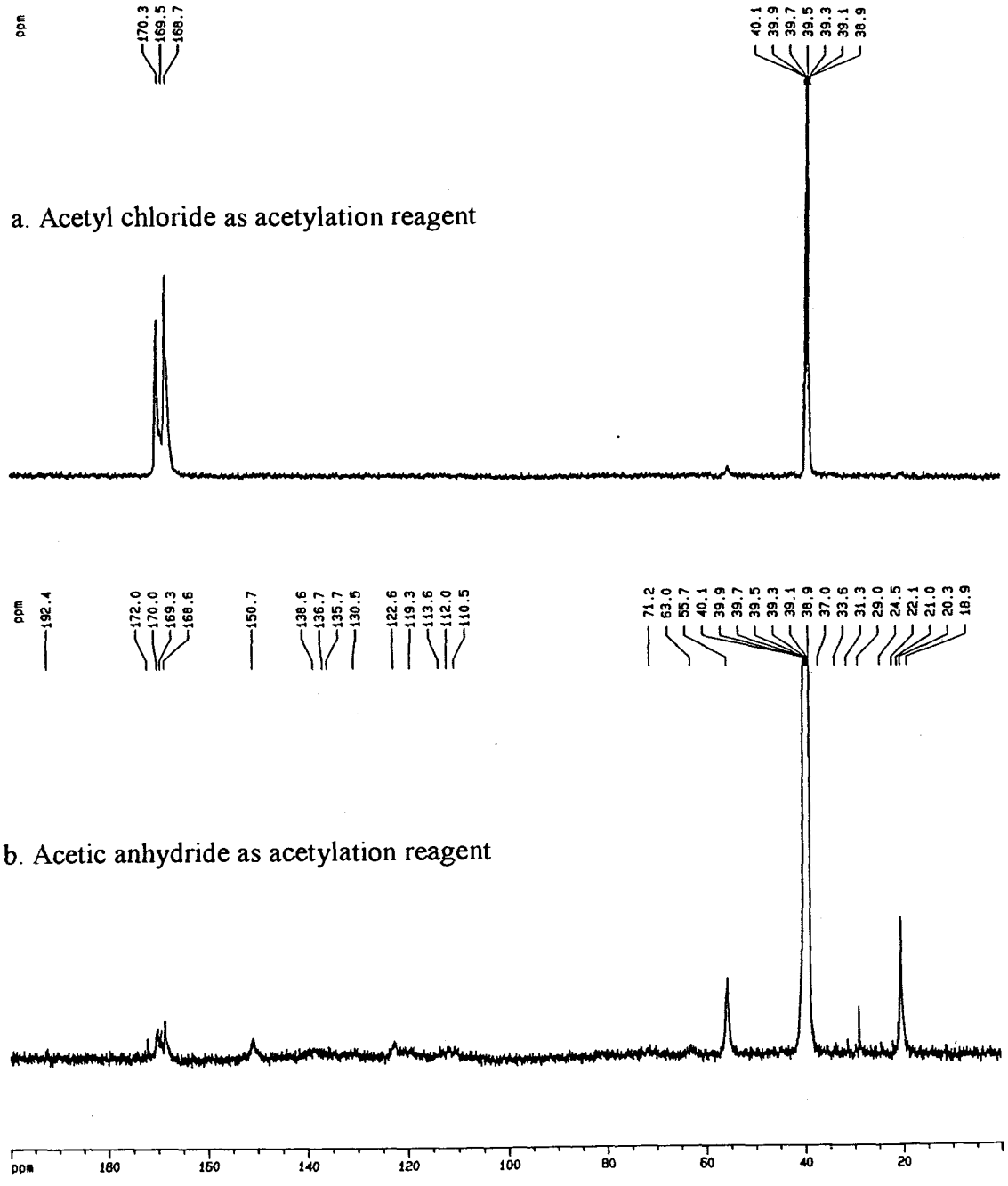

Figure 4.11. ^{13}C-NMR spectra of #2BS acetylated after dialysis filtration.

a. Acetyl chloride as acetylation reagent; b. Acetic anhydride as acetylation reagent.

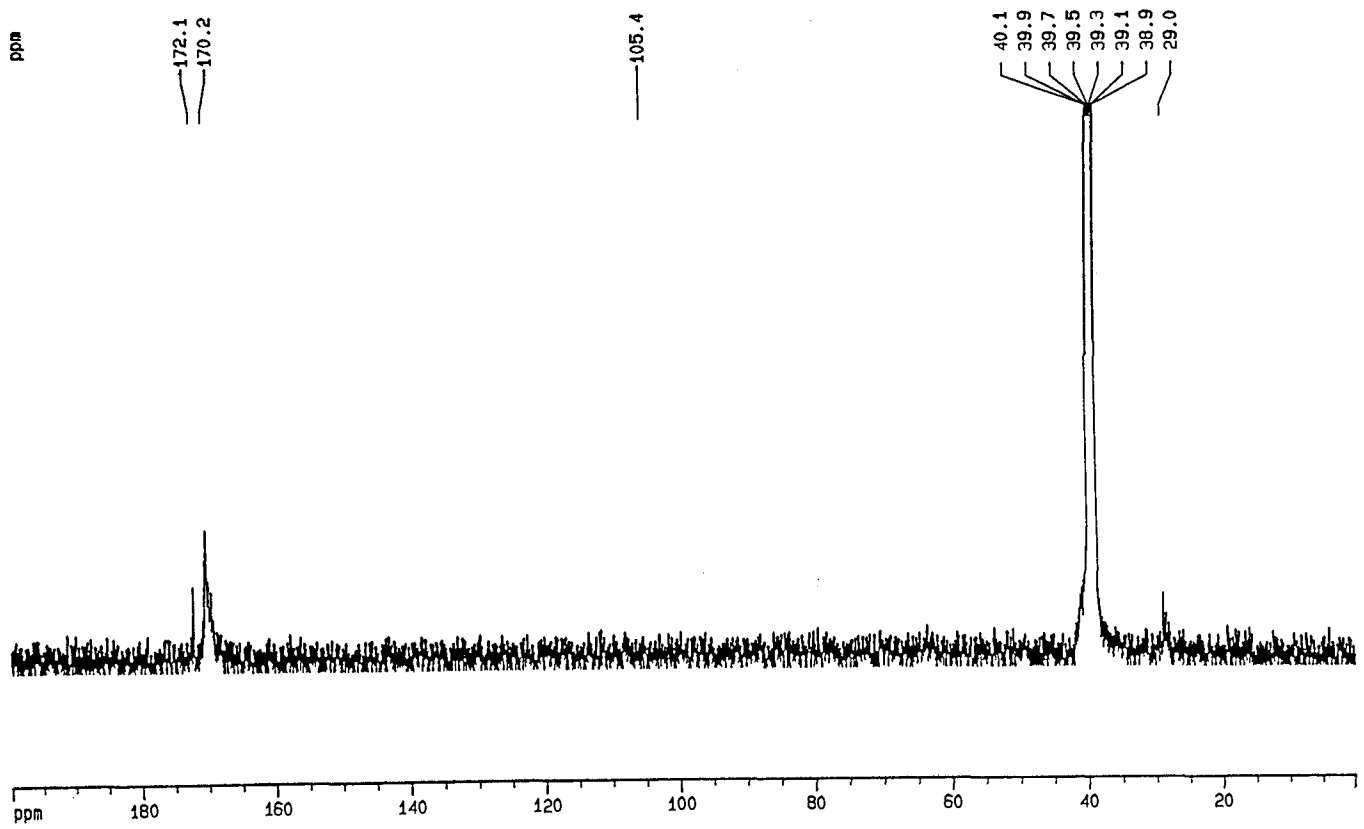

Figure 4.12. ^{13}C-NMR spectrum of CB acetylated after dialysis filtration. (DMSO-d_6 as solvent and internal standard. RD=12 s, PW=8.9, NS=315)

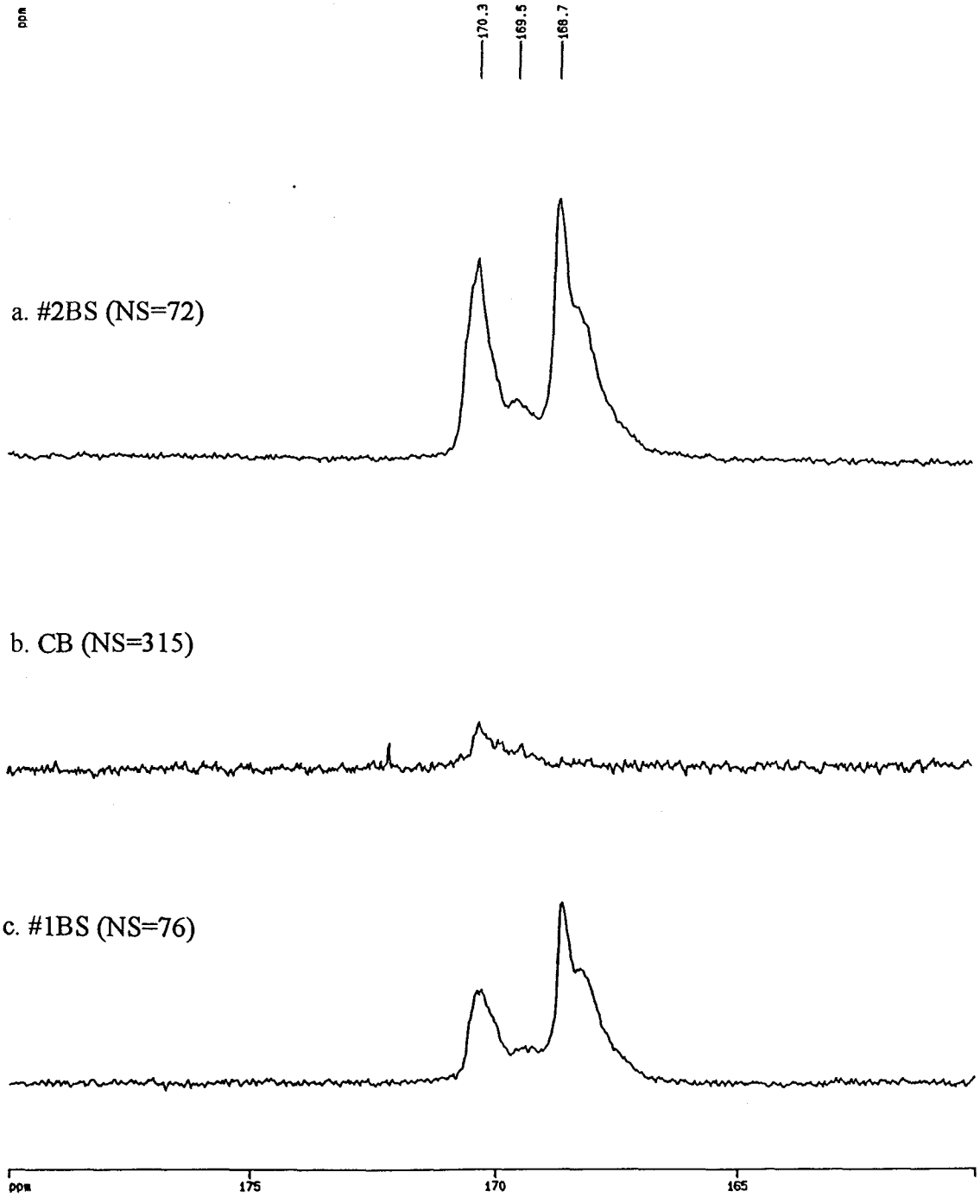

Figure 4.13. ^{13}C-NMR spectra of the filtrate lignins investigated in this work.

a. #2BS; b. CB; c. #1BS. All were acetylated by acetyl chloride after dialysis filtration.

were of limited values. No error analysis was performed on the data because the quantitative values were obtained from a single 13C-NMR spectrum for each sample.

However, from Table 4.13 and Table 4.14, some results can be obtained:

First, Alrich lignin had more carbonyl contents per aryl unit (2.93) but less contents per ary unit of [c-o] carbons (0.26), methoxyl groups (1.04) and aliphatic carbons (3.42) than #1BS (1.60, 0.80, 1.66, 5.85 respectively), which were close to what #2BS had (2.28, 0.91, 2.10, 5.08 respectively). This showed that the #1BS and #2BS were quite different from the normal softwood kraft lignin (Aldrich lignin), though #1BS and #2BS lignin were also obtained after kraft pulping. In fact, #1BS and #2BS lignin were more similar with the kraft black liquor lignins in the "Initial KBL" and "Final Permeate" [Wilson, 1987]. The causes of the differences may be due to different conditions of kraft pulping in the mill, and perhaps not only softwood chips were used.

Second, for the same prepared sample (#1BS), the NMR results were quite different under different NMR conditions (a: RD=12 seconds, PW=8.9; b: RD=0.5 seconds, PW=3.0). In order to have relatively accurate results, the same conditions of running the NMR should be maintained.

Third, there still were some differences between #1BS and #2BS, especially for the hydroxyl groups contents per aryl group. The primary hydroxyl content in #1BS (0.48 per aryl unit, 34% of the total hydroxyl groups) increased a lot to 0.67 per aryl unit, 41% of the total hydroxyl groups in #2BS. The secondary hydroxyl content was about the same in both lignins. Although the amount of phenolic content per aryl group was about the same (0.68 in #1BS and 0.64 in #2BS), the relative content dropped from 47% in #1BS to 39% in #2BS. This indicated that some reactions were occurring from

the #1BS stage to the #2BS stage. The reason could be due to some harsh chemical conditions, such as the pH value which in #1BS pulps was still very high (>12).

Table 4.15 also shows some useful results:

First, our data for the primary OH, secondary OH and phenolic OH in the Aldrich lignin were 0.28, 0.18 and 0.54 respectively, which fit very well with the data of Softwood Kraft (0.27, 0.18 and 0.53 respectively) from the reference [Orejuela and Helm 1996]. We know the Aldrich lignin we bought was softwood kraft lignin, so it means this method of NMR to quantify the relative contents of different hydroxyl groups in lignin and to identify the types of lignin (hardwood or softwood or mixed wood) is reliable.

Second, the data of #1BS (a) (NS=2696, acetylated without previous dialysis) were very close to the data of #1BS (b) (NS=76, acetylated after dialysis). This demonstrates that dialysis filtration does not have an effect on chemical structure, but it is very useful and necessary because it shortens the time of running NMR and a higher resolution of NMR spectra was obtained after dialysis.

Third, #1BS lignin has about the same ratios of the hydroxyl groups with the Organosolv mixed Hardwood [data from Orejuela and Helm 1996], which may show that the mill is using mixed chips from softwood and hardwood.

The last and most important point is that the structure of lignin had some change during the pulping and bleaching process, because the CB lignin had very different ratios of the three hydroxyl groups from the #1BS and #2BS lignin which had almost the same ratios. The ratio of phenolic OH dropped a lot from 0.65 in #1BS (B) to only 0.13 in CB, while the relative content of primary OH increased sharply from 0.27 in

#1BS (b) to 0.64 in CB. In fact, only primary OH was identified by NMR. This demonstrates that the phenolic OH is more active than primary OH. The result may be very useful for modeling the lignin structure after oxygen delignification.

Figure 4.14 showed the results of using dialysis filtration to deal with the #1BS filtrates. Most of the carbohydrates and wood extractives (99.6%) in the freeze-dried solids were eliminated after dialysis. Most of the lignins (67.6%) were still in the freeze-dried solids after dialysis, which agreed with the results of Ristolainen (1996) about the molecular-weight distribution (Md) of lignins during oxygen bleaching, 60.1% of lignins average molecular weight were larger than 1,000 (Table 2.2).

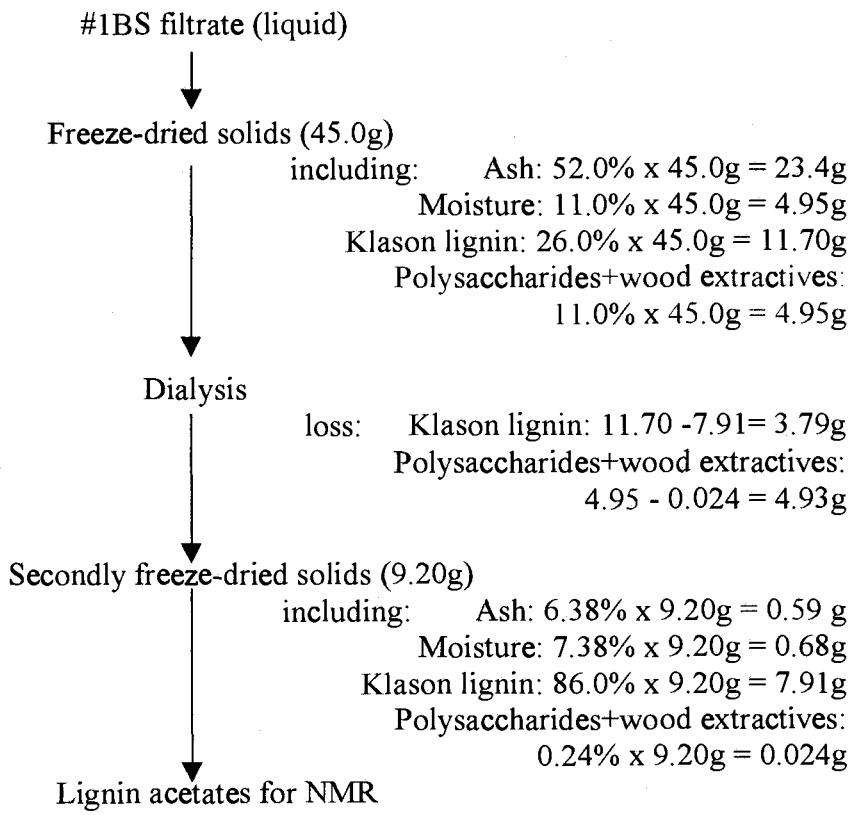

Figure 4.14. Components of #1BS before and after dialysis filtration

Table 4.13 Amounts of functional groups (acetylated by acetic anhydride)
(expressed as a percent of *total carbons in the spectrum)

Region (ppm-range)	Aldrich Lignin	#1BS (a)	#1BS(b)	#2BS	Ref (a)**	Ref (b)**
Carbonyl Carbons (190-165)	20 (20)	9 (10)	8 (11)	12 (11)	9	14
Aromatic Carbons (164-105)	41 (41)	32 (35)	20 (27)	31 (31)	36	25
[C-O-] Carbons (84-60)	2 (2)	4 (-5)	11 (7)	5 (2)	17	23
Methoxyl Groups (59-53)	7 (7)	15 (21)	21 (22)	17 (20)	6	4
Aliphatic Carbons (36-12)	23 (22)	32 (31)	33 (24)	27 (28)	22	26
Total Carbons (sum of above)	93 (92)	92 (92)	93 (91)	92 (92)	90	92

Notes: 1. * Total carbons in the spectrum determined by integrating from 190 ppm to 0 ppm.
2. ** Data from [Wilson 1987]. (a)--Initial KBL, (b)--Final Permeate.
3. Aldrich lignin,#1BS (a) and #2BS--NMR operational condition (RD=12 secs, PW=8.9),
#1BS (b)--NMR operational condition (RD=0.5 secs, PW=3.0)
4. All samples (not including Ref (a) and (b)) were acetylated by acetic anhydride after dialysis and freeze-drying.
5. The first numerical value for each sample results from manual phase correction of the spectra,
whereas the second value (in parentheses) was based on auto-phase correction of the spectra.
6. No reasonable results obtained for CB, Q and PO2 acetic anhydride acetates from NMR method.

Table 4.14 Amounts of functional groups (per aryl [C6C3] unit)

Functional Groups (ppm-range)	Aldrich Lignin	#1BS (a)	#1BS(b)	#2BS
Carbonyl (190-165)	2.93 (2.92)	1.60 (1.69)	2.53 (2.38)	2.28 (2.18)
Primary OH (171.3-169.6)	*0.41 (0.40)*	*0.48 (0.39)*	*0.65 (0.63)*	*0.67 (0.72)*
Secondary OH (169.6-168.9)	*0.21 (0.21)*	*0.27 (0.29)*	*0.34 (0.34)*	*0.33 (0.37)*
Phenolic OH (168.9-166.7)	*0.72 (0.70)*	*0.68 (0.92)*	*0.79 (0.84)*	*0.64 (0.78)*
*Total OH (171.3-166.7)**	*1.34 (1.31)*	*1.43 (1.60)*	*1.78 (1.81)*	*1.64 (1.87)*
[C-O-] Carbons (84-60)	0.26 (0.29)	0.80 (-0.88)	3.31 (0.98)	0.91 (0.36)
Methoxyl Groups (59-53)	1.00 (1.01)	2.76 (3.68)	6.24 ((4.90)	3.31 (3.78)
Methoxyl Groups (57.0-54.5)	*1.04 (1.01)*	*1.66 (1.83)*	*2.77 (2.72)*	*2.10 (2.38)*
Aliphatic Carbons (36-12)	3.42 (3.20)	5.85 (5.36)	9.91 (6.28)	5.08 (4.16)
Methyl (-CH3) (21.2-19.0)	*1.55 (1.45)*	*1.97 (2.14)*	*3.03 (2.94)*	*2.53 (2.87)*

Notes: 1. All data were derived from Table 4.13 using standard calculation way in reference [Robert 1992]
2. All samples were detected by NMR under operational conditions of RD=12 secs and PW=8.9, except that #1BS (b) was under NMR operational conditions of RD=0.5 secs and PW=3.0.
3. All filtrate samples were acetylated by acetic anhydride after dialysis and freeze-drying.
4. The first numerical value for each sample results from manual phase correction of the spectra, whereas the second value (in parentheses) was based on auto-phase correction of the spectra.
5. * Total OH was the sum of Primary OH, Secondary OH and Phenolic OH.
6. No reasonable results can be obtained for CB, Q and PO2 acetic anhydride acetates from NMR method.

Table 4.15 Peak area ratios for the hydroxyl groups of the lignin acetates
(samples were acetylated by acetyl chloride)

Region (ppm-range)	Primary OH (171.3-169.6)	Secondary OH (169.6-168.9)	Phenolic OH (168.9-166.7)
Aldrich Lignin (NS=79)	0.28	0.18	0.54
#1BS (a) (NS=2696)	0.23	0.07	0.70
#1BS (b) (NS=76)	0.27	0.08	0.65
#2BS (NS=72)	0.33	0.09	0.58
CB (NS=315)*	0.64	0.23	0.13
Softwood Kraft**	0.27	0.18	0.53
Organosolv mixed Hardwood**	0.27	0.10	0.62

Notes: 1.* Actually, only primary OH can be identified in NMR spectra of CB lignin acetates at 170.2ppm.

2.** These data were taken from the reference [Orejuela and Helm 1996].

3. All samples here except #1BS (a) were acetylated by acetyl chloride after dialysis.
   #1BS(a) sample was acetylated by acetyl chloride without dialysis before.

4. No reasonable peaks showed in the range of 171.3ppm-166.7ppm for Q and PO2 filtrate lignin acetates.

V. CONCLUSIONS

Conclusions from the major results are:

1. Different relative contents of the three hydroxyl groups, primary OH, secondary OH and phenolic OH were found in #1BS (0.27, 0.08, 0.65 respectively), #2BS (0.33, 0.09, 0.58 respectively) and CB filtrate (0.64, 0.23, 0.13 respectively) from TCF bleaching kraft pulp mill. #1BS and #2BS were filtrates from brown stock washing after kraft pulping. CB was the filtrate from brown stock after oxygen bleaching. This may suggests that the preferred reaction during oxygen bleaching was on the site of the phenolic OH, and that after the oxygen bleaching process, metal ions may then prefer to chelate with primary OHs because of their high content.
2. Differences between #1BS and #2BS appeared not only in the different relative contents of hydroxyl groups, but also in the amounts of other functional groups per aryl unit, although the differences were relatively low compared with those of the normal kraft lignin (Aldrich lignin) and those of kraft lignin in black liquors.
3. The two ways of lignin acetylation for NMR had their respective advantages and disadvantages. The method of acetylation with acetic anhydride could provide almost all functional group information in lignin but it was time-consuming and needed larger amounts of sample. On the contrary, the method of acetyaltion with acetyl chloride provided higher speed and greater resolution for NMR results but could only provide hydroxyl groups information in lignin.
4. The method of dialysis filtration helped to obtain better S/N NMR spectra and it did not change the chemical structure. It was a useful tool for lignin analysis.

5. The molecular-weight distribution of lignin in #1BS measured by the method of dialysis filtration was: 67.6% of lignin average molecular weight were larger than 1,000, which agreed well with the published data.
6. The optimum condition for lignin acetylation with acetic anhydride was under Condition E: 3ml pyridine, 1ml acetic anhydride, 2 ml methanol and 0.1N HCl, which yielded lignin acetates of 106.3%. The optimum condition for lignin acetylation with acetyl chloride was under Condition G: adding reagent acetyl chloride first, then the catalyst DMAP, which yielded lignin acetates of 103.8%.
7. No free monosaccharides were found in any of these six filtrate samples by thin-layer chromotography. All of them contained cellulose and hemicelluloses which were hydrolyzed into D-glucose, D-galactose, D-mannose, D-xylose and L-arabinose.
8. Catechol type compounds, dihydroquercetin, quercetin and catechin were found in #1BS and CB filtrates by thin-layer chromatography.

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APPENDIX

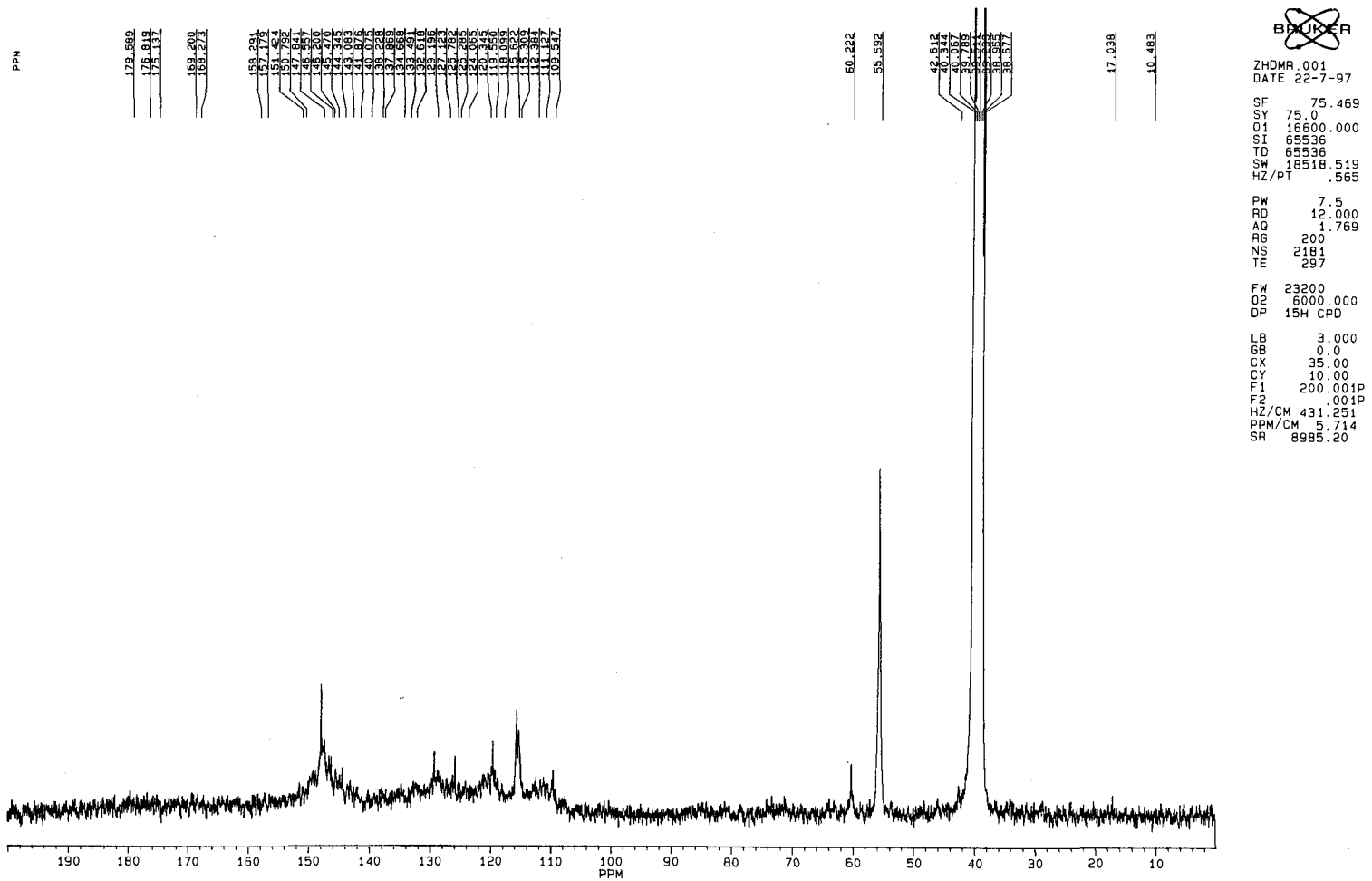

Figure A.1 ^{13}C-NMR spectrum of Aldrich lignin without acetylation.
(DMSO-d_6 as solvent and as the internal standard. RD=12 s, PW=7.5 and NS=2181)

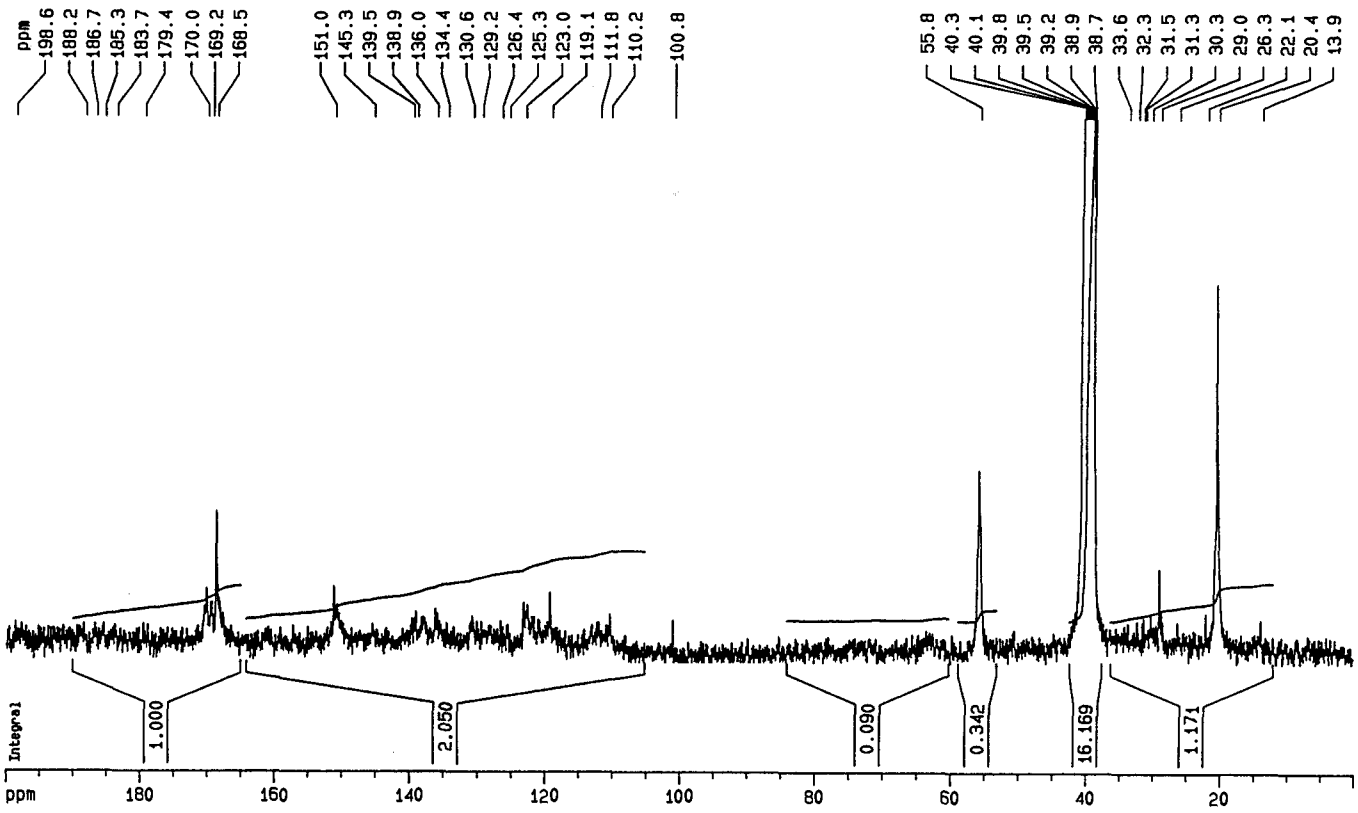

Figure A.2 Quantitative ^{13}C-NMR spectrum of Aldrich lignin acetylated under Condition E. (Acetic anhydride as acetylation reagent. RD=12 s, PW=7.5 and NS=2090)

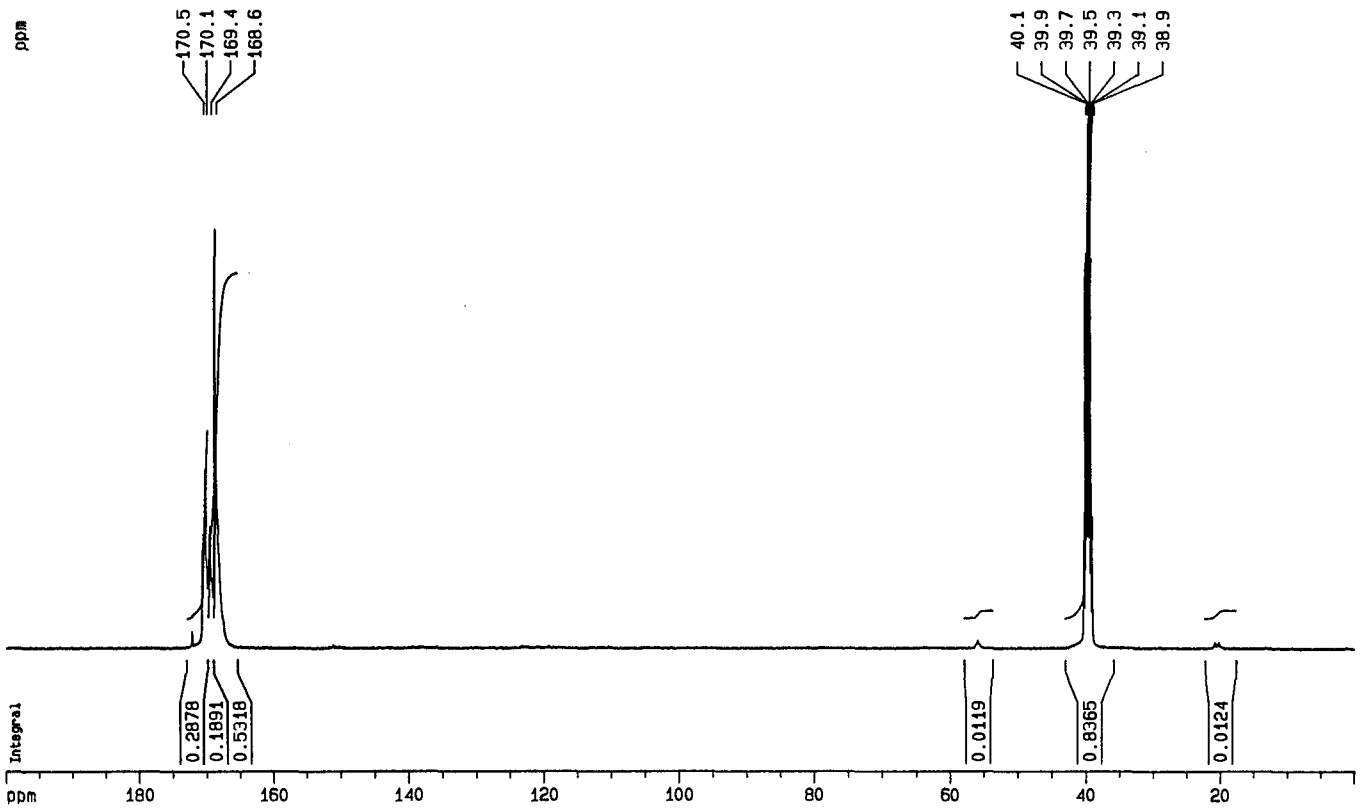

Figure A.3 Quantitative ^{13}C-NMR spectrum of Aldrich lignin acetylated under Condition G. (Acetyl chloride as acetylation reagent. RD=12 s, PW=8.9 and NS=79)

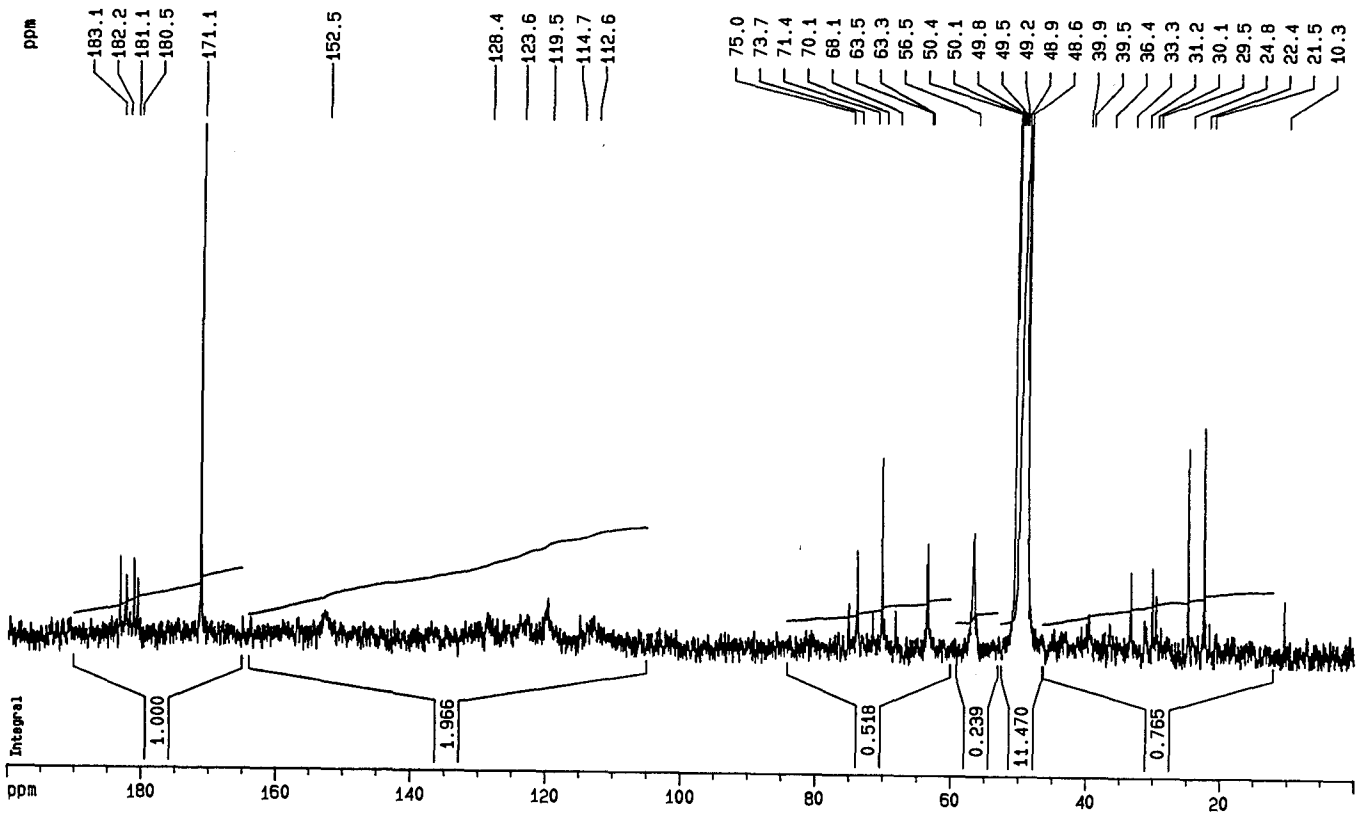

Figure A.4 Quantitative ^{13}C-NMR spectrum of #1BS lignin without acetylation and dialysis. (MeOH-d_4 as solvent and as the internal standard. RD=0.0s, PW=2.3 and NS=18237)

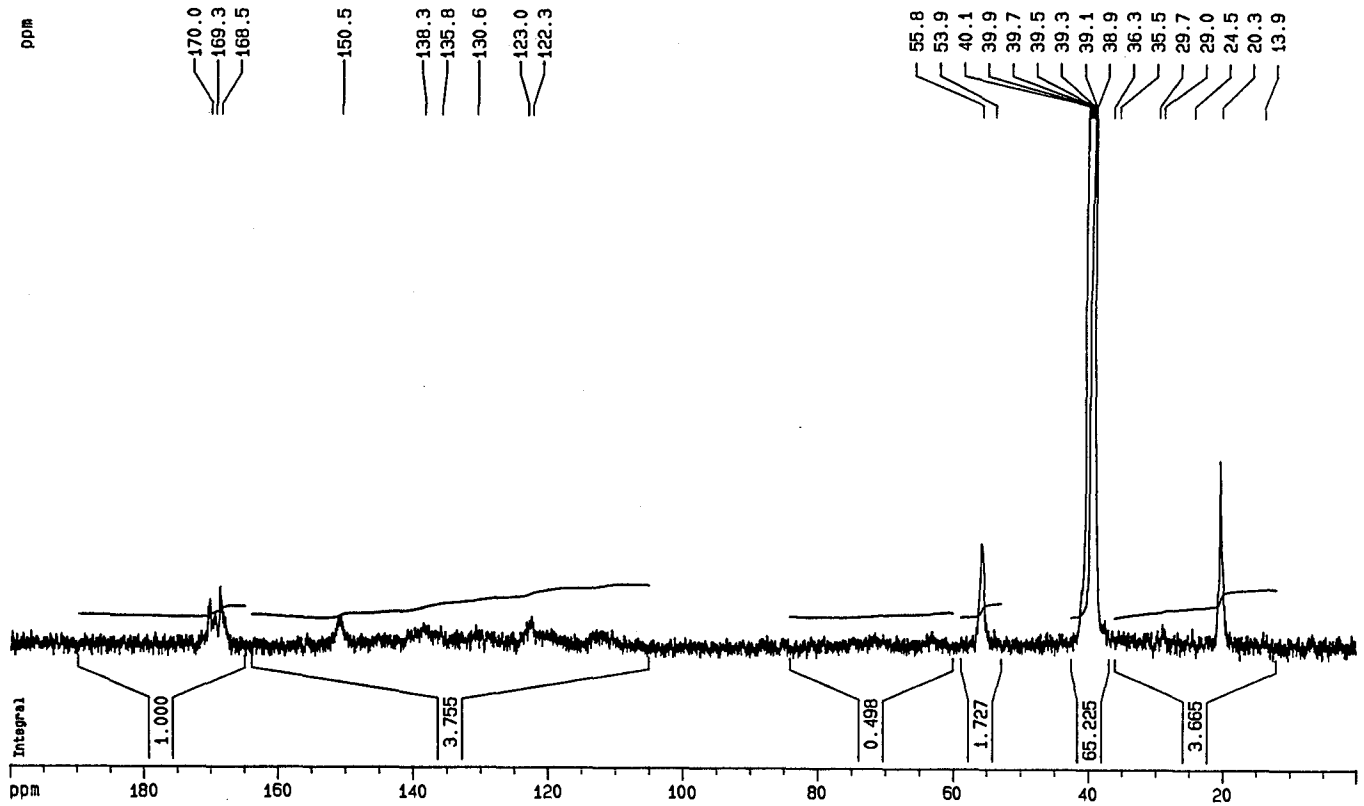

Figure A.5 Quantitative ^{13}C-NMR spectrum of #1BS lignin acetylated after dialysis. (Acetic anhydride as acetylation reagent. RD=12 s, PW=8.9 and NS=2764)

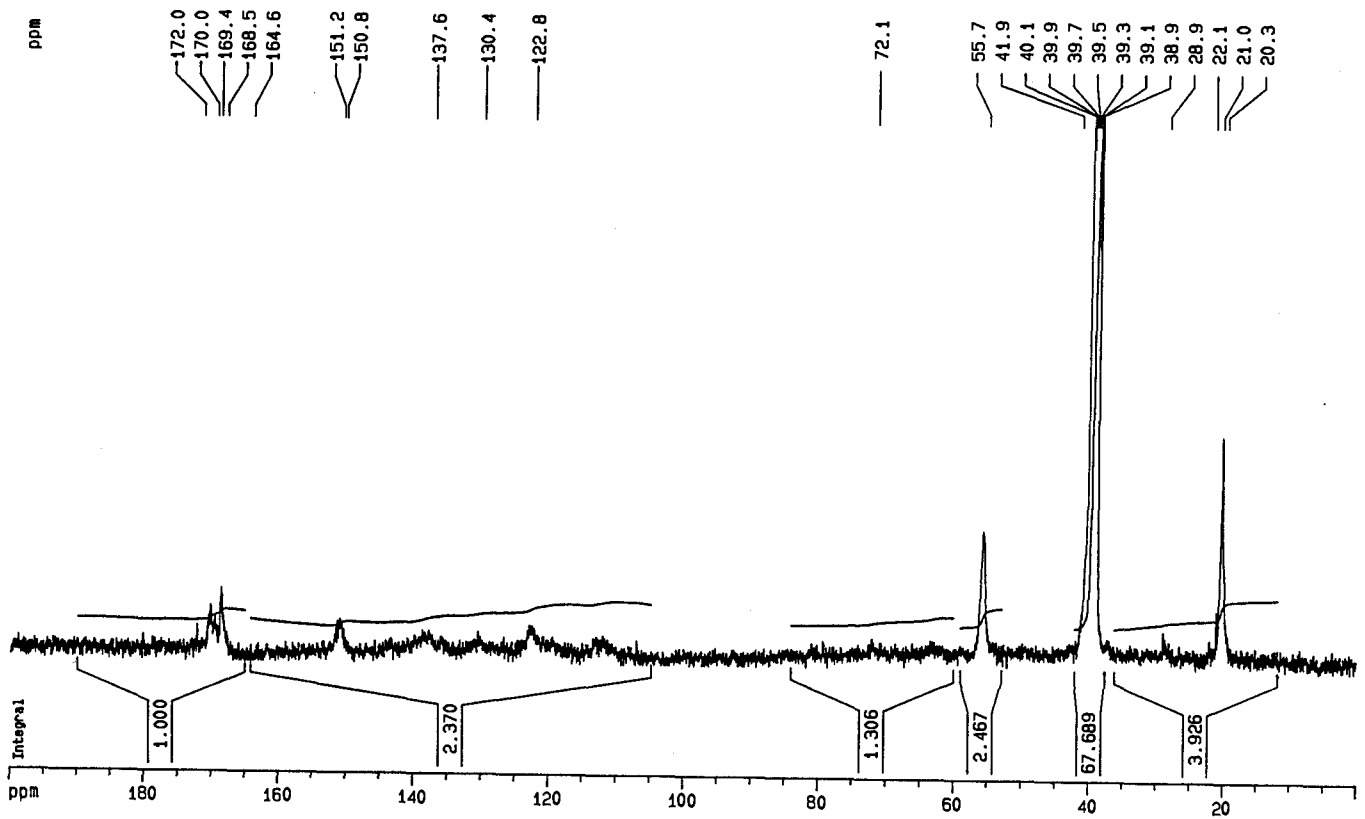

Figure A.6 Quantitative ^{13}C-NMR spectrum of #1BS lignin acetylated after dialysis. (Acetic anhydride as acetylation reagent. RD=0.5 s, PW=3.0 and NS=17633)

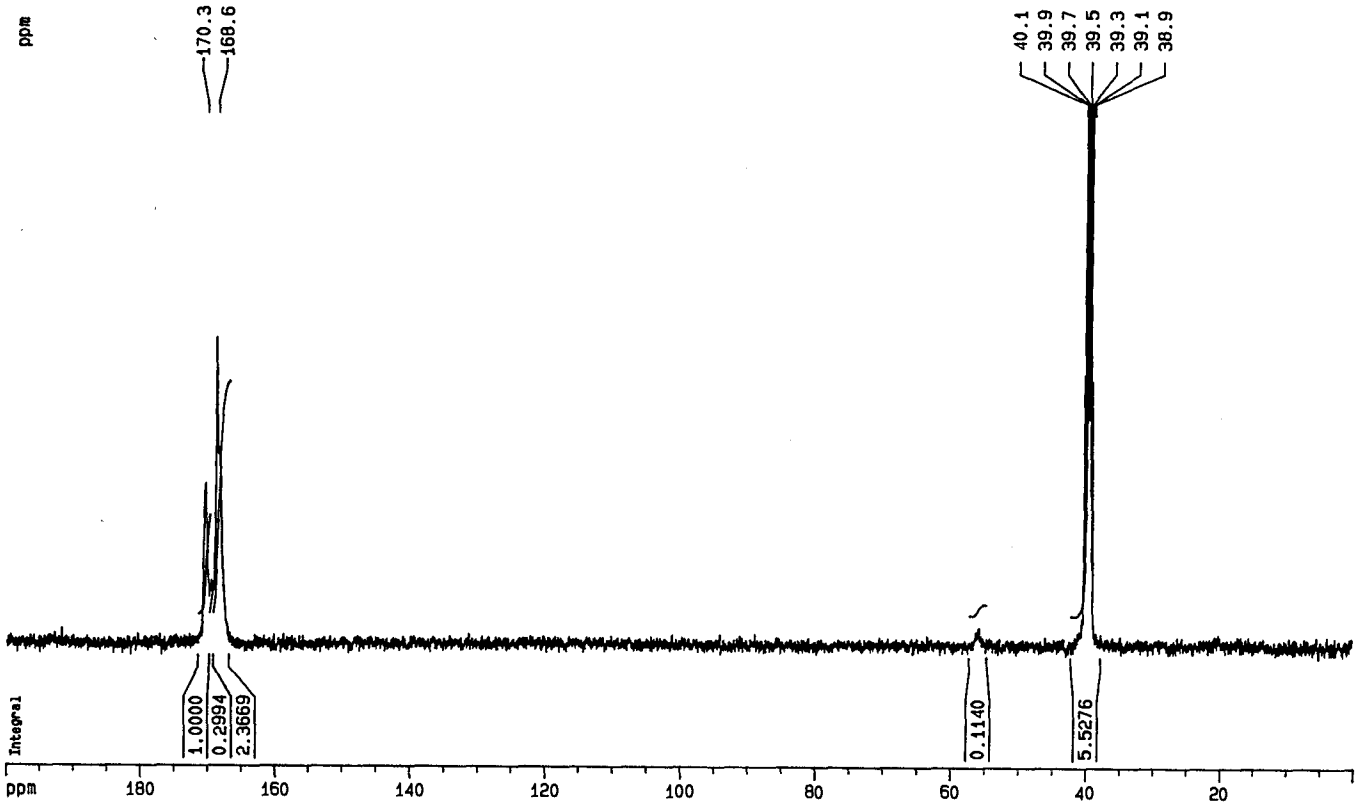

Figure A.7 Quantitative ^{13}C-NMR spectrum of #1BS lignin acetylated after dialysis.
(Acetyl chloride as acetylation reagent. RD=12 s, PW=8.9 and NS=76)

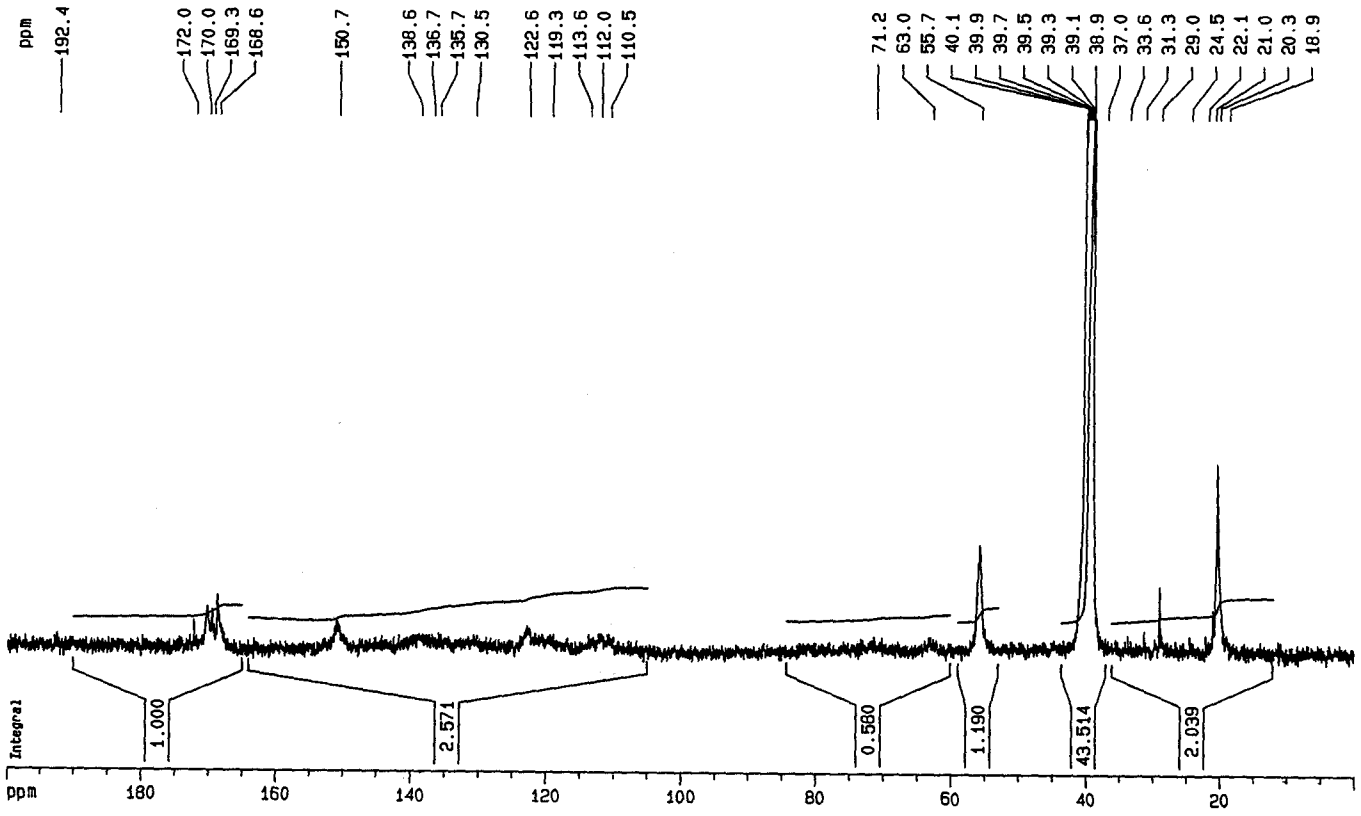

Figure A.8 Quantitative ^{13}C-NMR spectrum of #2BS lignin acetylated after dialysis.
(Acetic anhydride as acetylation reagent. RD=12 s, PW=8.9 and NS=2073)

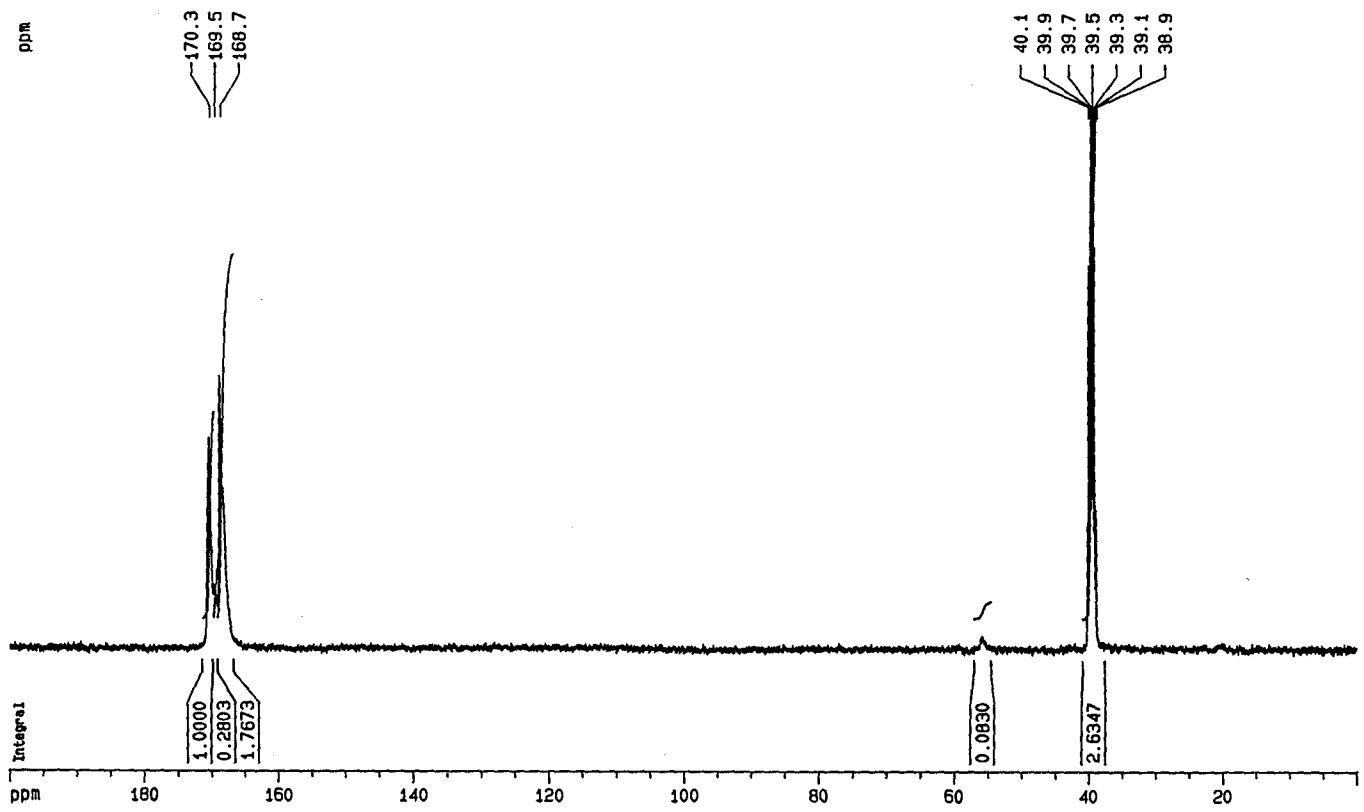

Figure A.9 Quantitative ^{13}C-NMR spectrum of #2BS lignin acetylated after dialysis. (Acetyl chloride as acetylation reagent. RD=12 s, PW=8.9 and NS=72)

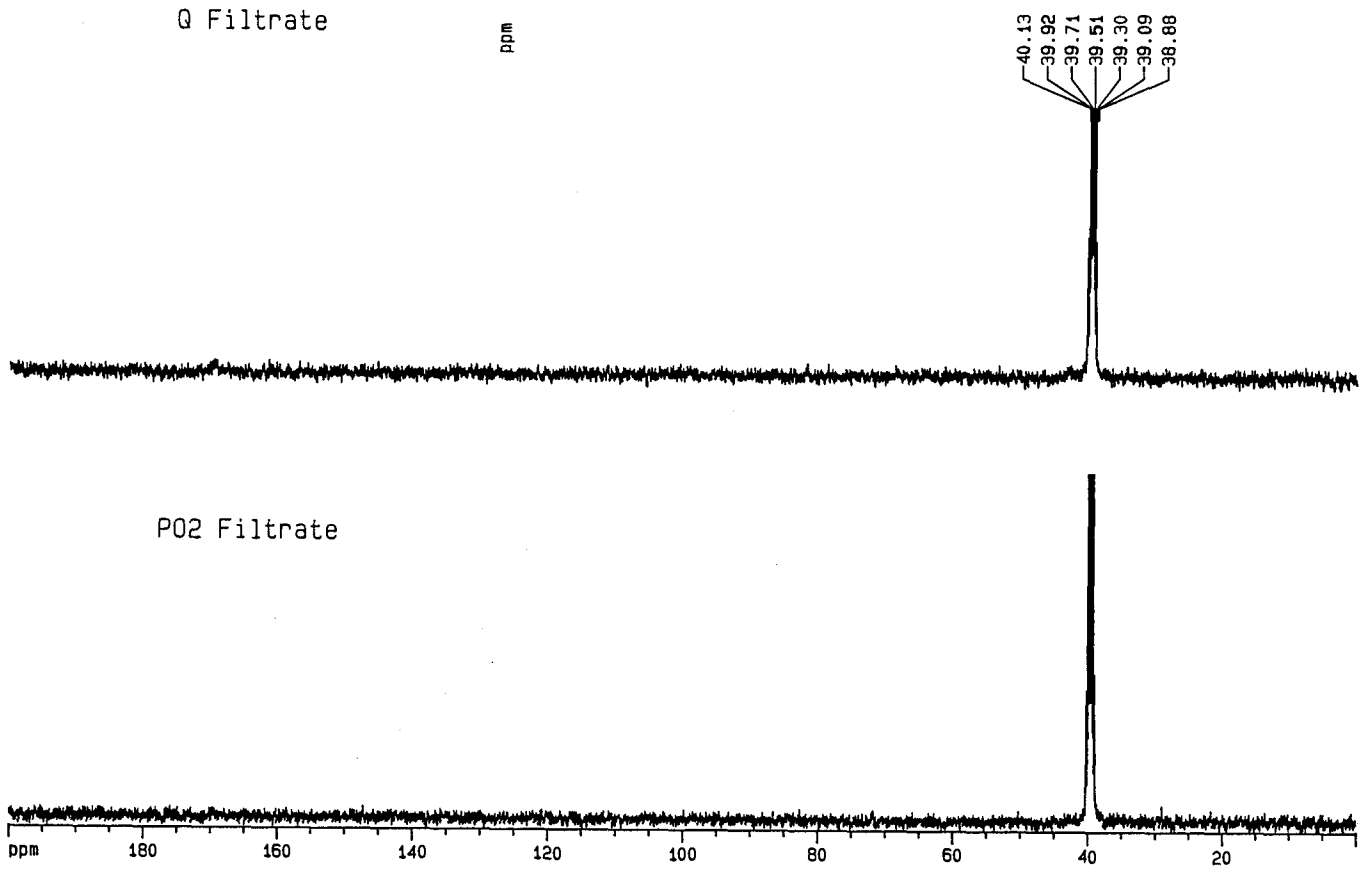

Figure A.10 ^{13}C-NMR spectrum of Q and PO2 lignin acetylated after dialysis.
(Acetyl chloride as acetylation reagent. RD=12 s, PW=8.9)