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ISOLATION AND CHARACTERIZATION  
OF DOUGLAS-FIR ORGANOSOLV LIGNIN

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## ABSTRACT

Granular water-insoluble lignins were isolated from a series of aqueous organic solvent (organosolv) cooks designed for pulping/saccharification of Douglas-fir sawdust. Among the factors affecting yield and characteristics of the isolated organosolv lignins, only cooking time (5-20 minutes) and concentration of acid catalyst (0-0.1N HCl) were investigated as cooking variables. Cooking temperature (200°C) and solvent composition (acetone/water=60:40) were held constant.

It was learned that the acidified organosolv cooking system is far more efficient in delignification and saccharification than aqueous acid hydrolysis under identical conditions. In organosolv cooking, simultaneous dissolution of lignin and sugars occurs in the cooking liquor, allowing continued and total dissolution of the wood constituents. In the present study, only the water-insoluble lignin fraction was isolated and analyzed.

An almost quantitative recovery of the precipitable lignin was accomplished by evaporation of the organic solvent from the spent liquor, followed by removal of sugars dissolved in the aqueous solution and reprecipitation of the crude lignin into water. To eliminate the interference from hydrogen bonding and unconjugated carbonyl group in the isolated organosolv lignins, acetylation or reduction was carried out before the lignin samples were characterized. The resulting lignin samples were found to be completely free of carbohydrate contaminants.

Both cooking time and acid concentration were found to have a profound effect on the yield of lignin fractions, and chemical and macromolecular properties of the lignin molecules due to two competing

reactions, hydrolytic depolymerization and recondensation. These reactions take place simultaneously in the cooking liquor during organosolv cooking.

The balance between these two reactions is believed to be responsible for not only the content of functional groups, as revealed by nuclear magnetic resonance, infrared and ultraviolet spectral analyses, but also the size of lignin molecules, as measured by gel permeation chromatographic and scanning electron microscopic analyses of the isolated organosolv lignins.

The functional group contents, determined by elemental and spectral analyses, were found to be 0.86-0.97 methoxyl, 0.20-0.49 aromatic hydroxyl and 0.68-0.99 aliphatic hydroxyl groups per  $C_9$ -unit of the organosolv lignin molecules. It was also noted that 63-68% of aromatic nuclei have condensed forms with carbon-carbon linkages, having only two hydrogens on each guaiacyl nucleus.

The organosolv lignins were found to have much lower molecular weights than those of protolignin in wood. Typical values of the number average molecular weight of the isolated lignins ranged from 823 to 1,144. The low molecular weight values are due to degradation reactions during the cooking by cleavage of aryl-alkyl linkages of lignin molecules. The particle size of the spherical precipitated lignins ranged from 25 to 500 nm.

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## 1. INTRODUCTION

The rising cost and expected shortage of crude oil and natural gas, which are the main raw material sources for the organic chemical industry, have stimulated search for alternate chemical feedstocks. Lignocellulosic materials, such as wood and straws which represent the largest renewable bio-resources on the earth, are considered to be one of the most important near-term substitutes for oil and natural gas (74,75,78,79,154).

During the past several years, considerable attention has been given to chemical utilization of wastes from the chemical pulping industry, and the literature is abundant with suggestions for the recovery of by-products from spent liquors (74,118,129,131,133). Spent cooking and wash liquors, recovered from chemical pulping processes, contain practically all the non-cellulosic wood constituents such as lignin, hemicelluloses and minor constituents of wood.

Based on data of the primary production of wood and other annual and perennial land plants, it can be calculated that the worldwide annual production of lignins, which represent about a quarter of lignocellulosic materials, is about 20 billion tons (154). According to FAO information (154), the total amount of lignin obtained either as alkali lignin or as liginosulfonic acid is approximately 40 million tons annually. About 70 to 80% of this amount is burt for heat recovery in the pulp mills (131).

Although this transformation of lignin into heat is an economic method of disposal of the waste liquor as far as the pulping processes are concerned, it seems like a wasteful manner of treating such a valuable chemical raw material. With marketable lignin preparations, the chemical value of lignin is greater than its fuel value, and lignin becomes an important revenue generating by-product of pulping (118,133, 154).

At the present time, lignin is not only used as starting or intermediate raw material for monomeric organic chemicals, but is also widely used in adhesives, binders, dispersants, and as extenders for resins and rubbers, emulsion stabilizer, grinding aid, boiler water ingredient and ion exchange resins (74,78,132). The features of lignin important for chemical utilization are its aromatic character and covalent carbon-carbon bonding (78).

Although biomass processing holds promise for generation of chemical feedstocks for both chemical and processing industries, only few economic processes are known to produce organic chemicals from lignin today. Difficulties arise mainly due to the condensed and contaminated state of industrial lignins (154). Pulping processes, which allow separation of the main components of lignocellulosic materials in such a manner that lignin can be obtained free of contaminants (especially without sulfur-substituents and sugar residues) and in a less condensed state, may offer better potential for its utilization as a raw material for the chemical industry.

With increasing provisions by law and public concern over the environmental impact of pulp mill effluents, development of new non- or less-polluting pulping processes is also one of the most important prob-

lems to be solved for the pulping industry (112).

A new organosolv pulping process, which may provide some of the answers to these problems, have been worked out in the pulp and paper laboratory of the Faculty of Forestry over the last few years. The present study is concerned with isolation of lignin from the organosolv cooking and characterization of the isolated lignin. In earlier studies, Chang and Paszner (36,37) concentrated on describing the processes which lead to maximum sugar yields and the total dissolution of aspen and Douglas-fir woods in organosolv cooking liquors, but no attention was paid to the quality of the dissolved lignin. Thus, the objectives of this study were:

- 1) to isolate the organosolv lignin from Douglas-fir sawdust which comprises a substantial portion of the wood raw material supply to many pulp mills in the Pacific Northwest,

- 2) to investigate the effect of various cooking conditions on chemical and macromolecular properties of the isolated lignin, and

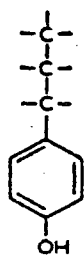
- 3) to draw inferences from these data as to the sensitivity of organosolv lignin to degradation, dissolution and recondensation as well as molecular condensation with other sugars or their derivatives formed during the high temperature cooking process.

## 2. LITERATURE REVIEW

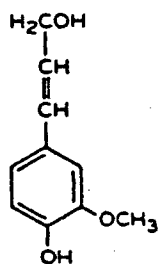
### 2.1 Definitions

Lignin: Lignin has never been specifically defined because it is not a definite chemical entity, and its polymeric chemical structure has not been fully elucidated.

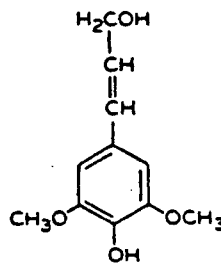
Lignin, however, is generally considered to be a system of a thermoplastic tridimensional polymer in which  $C_9$ -phenylpropane units(I), linked together by C-O-C and C-C linkages appear to be the basic units (26,68,131). The concept of lignin derived from an enzyme-initiated dehydrogenation polymerization of a mixture of three primary precursors, namely coniferyl alcohol (II), sinapyl alcohol (III) and p-coumaryl alcohol (IV), is now well established (65,66,67).



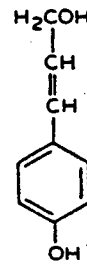
(I)



(II)



(III)



(IV)

Organosolv Lignin: Lignins obtained by procedures of extraction by means of organic solvents, usually in the presence of a catalyst, have been called "organosolv lignins" (131). These organosolv lignins are soluble in organic solvents employed as well as other solvents generally known as lignin solvents.

## 2.2 Distribution of Lignin

Until early in the 19th century, wood was considered to be a single chemical entity. This belief was held until Payen (130), in a paper published in 1838, showed that wood is composed of several components including a fibrous material, cellulose and an "encrusting material" which was later termed as "lignin". In plants, the first indication of lignification can be seen at the time of the onset of the wall thickening phase (163,164). In 1965, Wardrop (164) showed that the first deposition was at the cell corners within or just inside the primary wall. Following this initial deposition at the cell corners, lignification then extends along the middle lamella and into the secondary wall (63,162).

Lignin distribution in wood has been of considerable interest for both theoretical and practical reasons. According to Ritter (140), lignin exists in wood in two forms, namely, the 'middle lamella lignin', and the 'cell wall lignin', implying differences not only in location but accessibility, composition and possible association with other cell wall components, mainly hemicelluloses.



The results of most recent workers in the field were reviewed by Sarkanen and Hergert in 1971 (148), and Côté in 1977 (43). In 1965, Berlyn and Mark (19) showed that less than 40% of the total lignin in softwood is in the middle lamella, most of it being found in the secondary wall of coniferous tracheids. This new position has been supported by more recent evidences (18,44,45,69,128).

Using ultraviolet and fluorescence optics, Frey-Wyssling (69) demonstrated the **uniformity** of distribution of lignin across the secondary cell wall as well as across the middle lamella. Sacks *et al.* (142) suggested that a greater portion of lignin is concentrated in the compound middle lamella of maple, and that the lignin network in the secondary wall appears less dense than in softwoods. The same conclusion has been drawn from ultraviolet investigation on hardwood tissues (101).

More recently, in 1978 Gratzl and his co-workers (143) developed a new method to determine lignin distribution by using energy-dispersive X-ray analysis of brominated wood sections coupled with scanning electron microscopy. The data from the corresponding peaks of brominated wood samples show that the lignin concentration is very high in the middle lamella region, decreases toward middle part of the cell wall, and slightly increases again near the lumen. The overall lignin distribution is in agreement with the results of **earlier** microscopic studies (128,142) on lignin skeletons created by the removal of carbohydrates with hydrofluoric acid. Lignin distribution across the cell wall has important implications in delignification and fiber separation from lignocellulosics affecting both fiber yield and cellulose purity of pulps.

### 2.3 Formation and Chemical Structure of Lignin

The plant lignins of interest can be divided into three classes, which are commonly called (i) gymnosperm or softwood lignins, (ii) angiosperm or hardwood lignins and (iii) monocotyledonous angiosperm or grass lignins (131). According to several earlier investigations (65,158,161), it was known that the most primitive land plants, as well as softwoods, have lignins in which guaiacylnuclei or coniferyl alcohol (II) predominates whereas in hardwood lignins, both coniferyl alcohol (II) and syringyl nuclei or sinapyl alcohol (III) are present even though there are some exceptions to this generalization (54,55,73). Grass or annual plant lignins generally are polymers of synapyl alcohol (III) and p-hydroxyphenyl propane (I) units.

In common with all other organic plant constituents, lignin must be derived **ultimately** from carbon dioxide. Although the complete scheme of biogenesis of lignin in the tree is still far from totally known, there appears to be little doubt that lignin originates from the carbohydrates which are formed from atmospheric carbon dioxide by the process of photosynthesis (87,131). Thus, the first phase of lignin **biogenesis** involves the conversion by living plant cells of non-aromatic precursors such as carbohydrates into compounds containing benzenoid type rings which becomes a part of the basic structure of lignin. As the first clues to this conversion, around 1955, Davis, Sprinson, and their co-workers (47,103,157) demonstrated that radiation-induced

mutants of the bacterium Escherichia coli, which lacked enzymes necessary for aromatic ring formation, accumulated in growth-medium compounds that have proved to be obligatory intermediates in the conversion of sugars to benzenoid compounds. The so-called Davis-Sprinson pathway (102,131), as understood at present time on the basis of more recent findings (28,29,50,71,152), for the biosynthesis of the aromatic precursors of lignin is pictured in Fig. 1.

D-Erythrose-4-phosphate (V) and 2-phosphoenolpyruvic acid (VI), both formed from glucose combined to form an intermediate phosphate (VII), which then forms the cyclic 5-dehydroquinic acid (VIII). The biosynthesis then proceeds through the obligatory intermediates, 5-dehydroshikimic acid (IX) and shikimic acid (X). On the basis of tracer and enzyme studies, Brown (28,29) proposed the pathways from shikimic acid (X) to the three lignin-monomers (II,III,IV). He pointed out the fact that not all reactions in this pathway occur in all species. The scheme indicates that all lignified plants possess the enzymes necessary to carry out the reactions in the sequence. It should be emphasized that lignification pathways other than those shown in Fig. 1 may also exist.

The second phase of lignin biosynthesis involves the dimerization of the monomer precursors (II,III,IV) and the continued growth of molecule by the oxidative polymerization. The efforts to clarify the structures of the different types of lignin have resulted in a detailed picture of the various modes in which the phenylpropane units

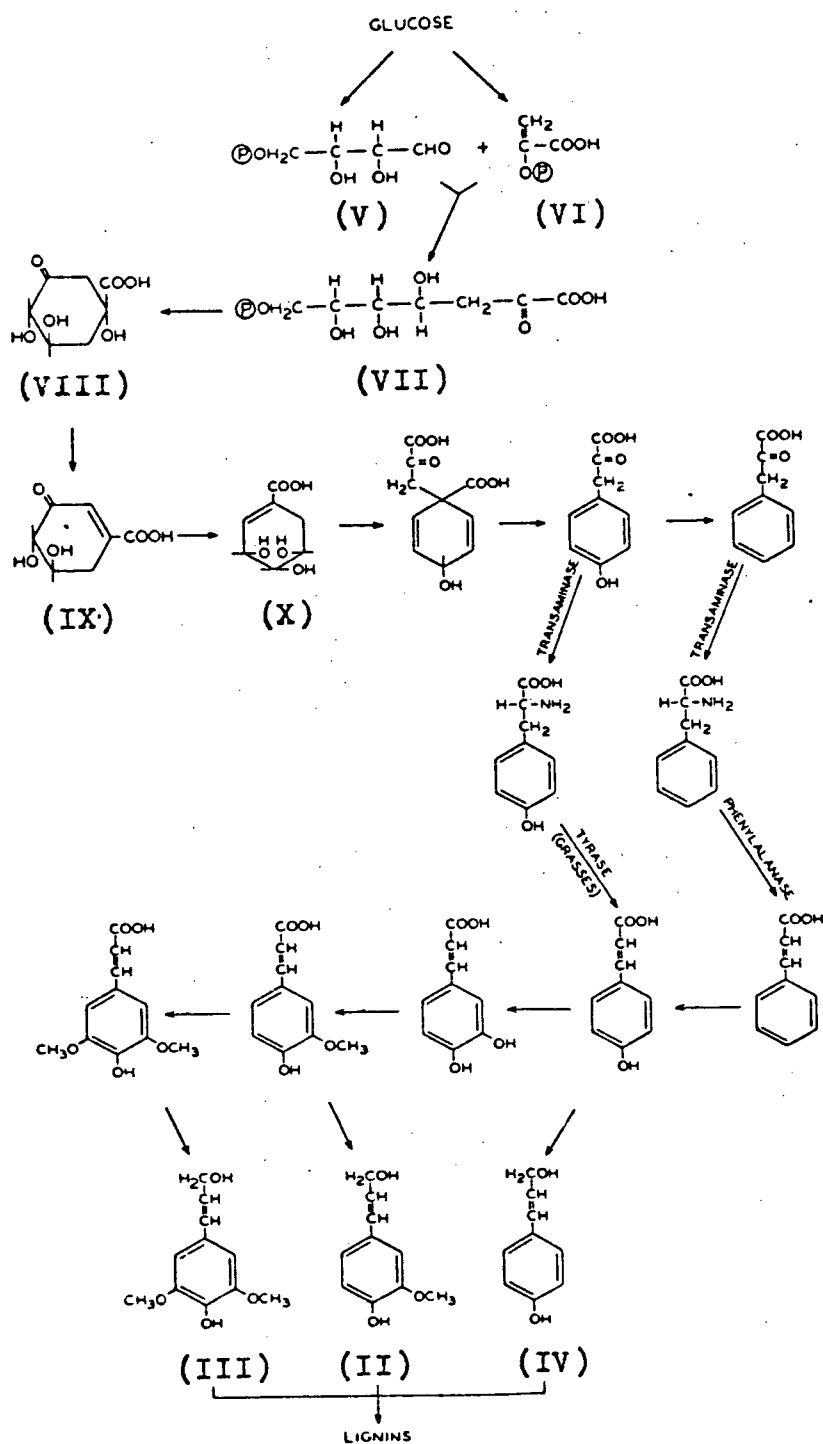


Figure 1. Pathways for the conversion of glucose to lignin-monomers in plant (131).

(I) are linked together in the polymer. This problem has been investigated by two general methods, degradation and synthesis of lignin.

As early as 1933, Erdtman was successful in dehydrogenating a number of monomer model compounds to dimeric products (51,52). He suggested that lignin is formed in nature by an oxidative polymerization of phenolic precursors. Freudenberg and co-workers (67,68) showed that enzymes with laccase and peroxidase activities are probably responsible for dehydrogenation.

Freudenberg formulated the following mechanism for initial reactions of the dehydrogenation polymerization of coniferyl alcohol (II) as shown in Fig. 2 (2).

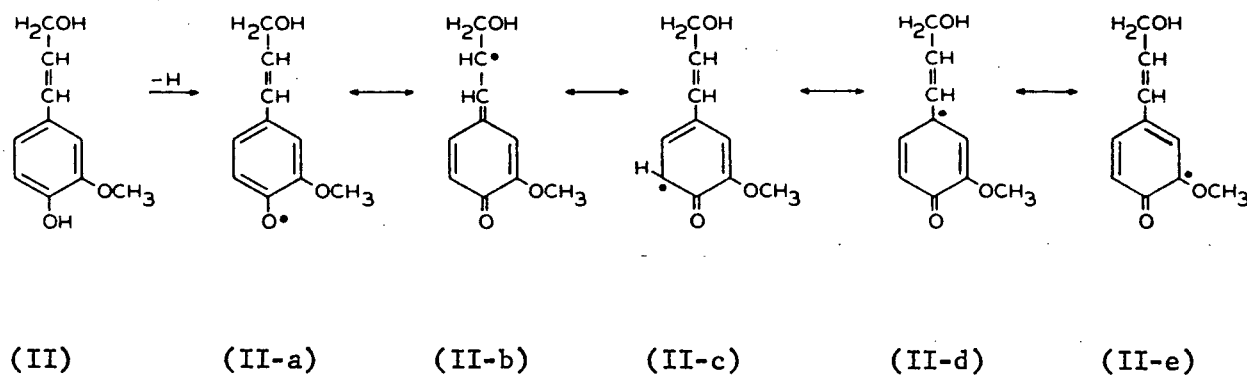


Figure 2. Dehydrogenation of coniferyl alcohol (II).

The enzymatic dehydrogenation is a one-electron transfer resulting in the formation of a resonance-stabilized phenoxy radical, dehydrogenated from coniferyl alcohol (II). Stabilization of the radical occurs by coupling to another radical in any of the positions of the unpaired electron given in resonance structures (II-a, II-b, II-c, II-d, II-e). These mesomeric radicals then intercombine. The continued growth of the molecule will predominantly take place by what has been called "end-wise" polymerization (145). The process is illustrated by an example in Fig. 3 where a coniferyl alcohol radical in its resonance form (II-b) is attached by  $\beta$ -0-4 coupling to an end group radical (II-a). The result of this coupling will be a quinonemethide (XI) which will react further by addition of a molecule of water to give the ether structure (XII).

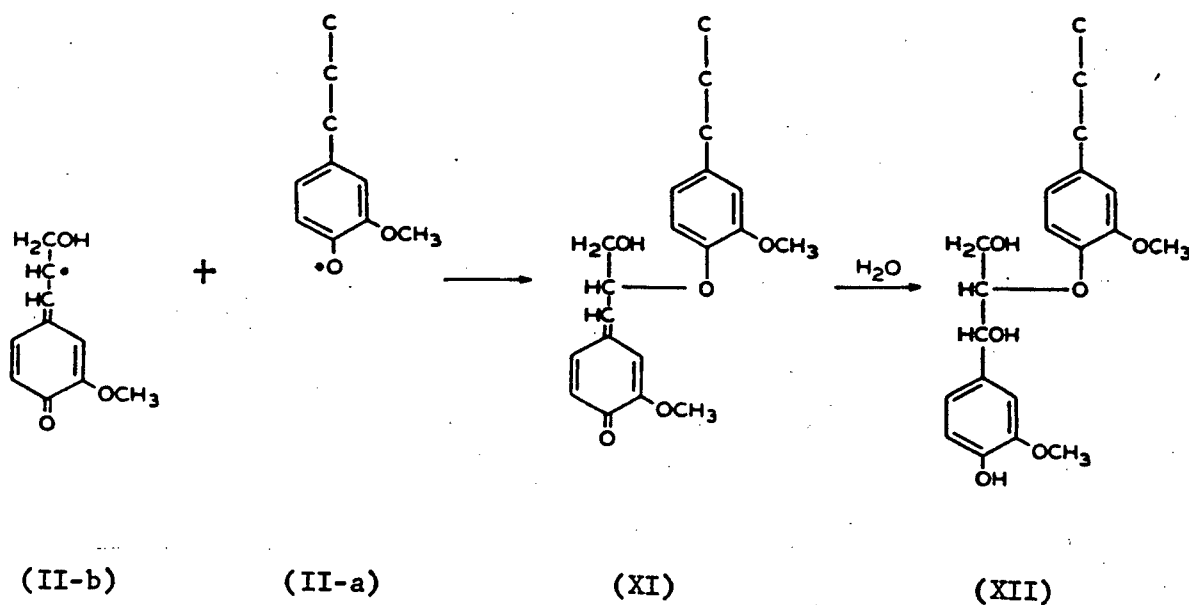


Figure 3. "End-wise" polymerization (2).

The formation of dimers is followed by further polymerization to tetrameric and high molecular weight aggregates. A great many formulae for lignin polymers have been proposed over the years (3,24,51,52,64,65,67).

In 1965, Freudenberg (65) proposed a structural formulation of Fig. 4 as a constitutional model for softwood lignin based on enzymatic dehydrogenation experiments. The resulting formulation containing 18  $C_9$ -units are interlinked in a fashion corresponding to the biochemical growth of the naturally occurring lignin molecule. It represents only a fraction of a lignin molecule. More recently the prominent substructures of spruce lignin were collected in a scheme (Fig. 5) comprising 16  $C_9$ -units (2,53).

In 1974, Glasser and Glasser (73) developed a mathematical simulation of reactions with softwood lignin building units by computer. The simulated structure of softwood lignin molecule, which consists of 81  $C_9$ -units, involves rather large globular configurations that are difficult to represent on a two-dimensional scale. The structural sketch depicted by them shows that 15% of the  $C_9$ -units are derived from p-coumaryl alcohol (IV), 79% from coniferyl alcohol (II) and 6% from sinapyl alcohol (III). The proportions of the three monomers (II,III,IV) involved in the copolymerization process vary in different woods (53) and even in different morphological parts of the wood, thus giving rise to the different lignins (2). These studies point out the potential difficulties in obtaining uniformly depolymerized lignins.

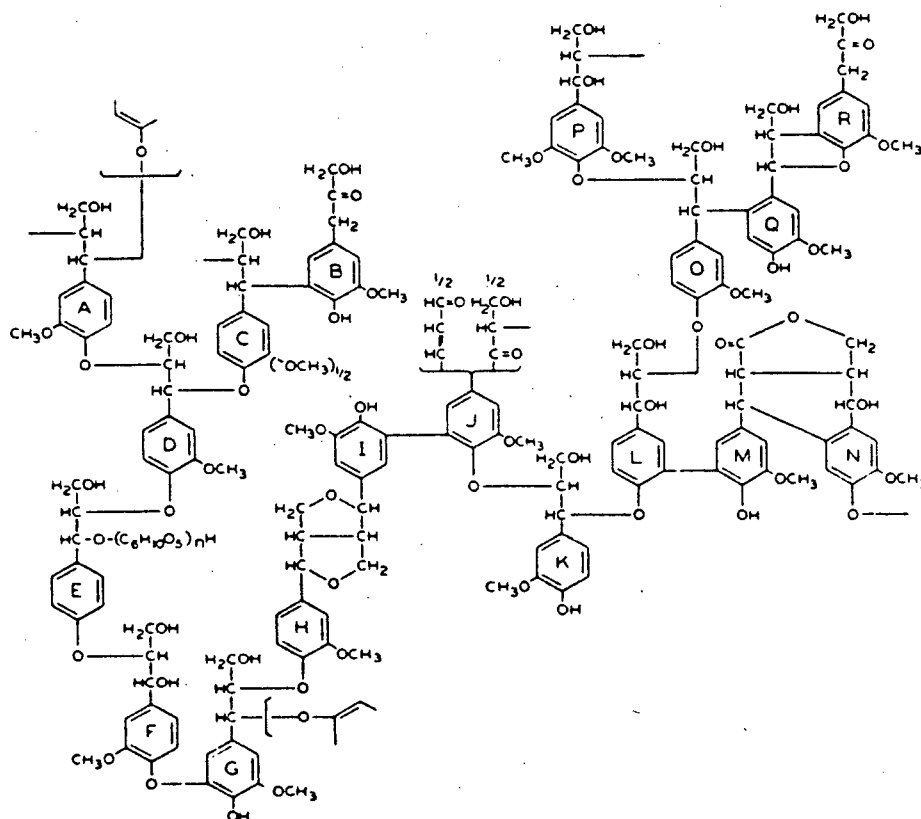


Figure 4. Freudenberg's formulation of softwood lignin (65).

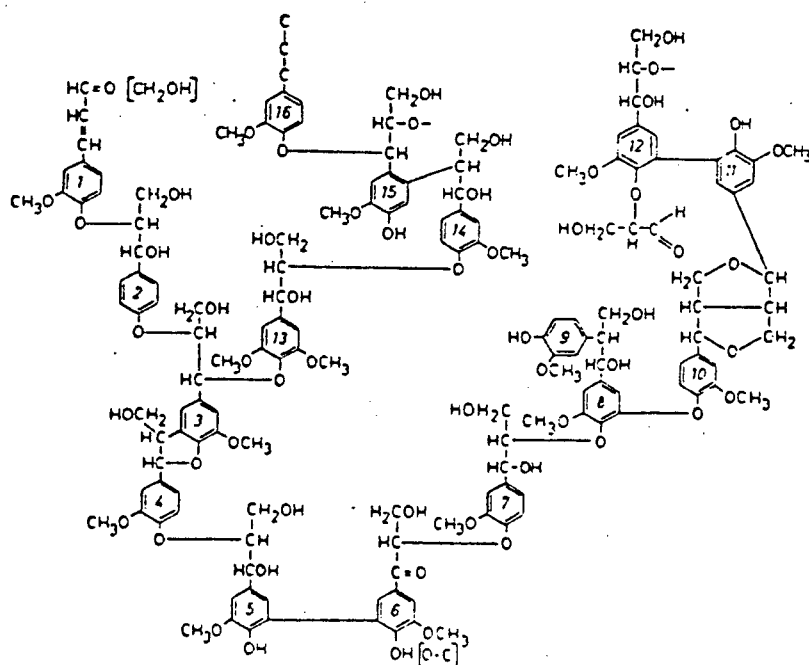


Figure 5. Prominent structures in Spruce lignin (2).



## 2.4 Isolation of Lignin

No method has yet been developed for the isolation of the protolignin in its entirety originally present in the wood. Many common methods of isolation cause fundamental changes in the lignin structure and the lignins obtained are different in many physical and chemical properties from the native lignin in wood (131).

In order to isolate lignin from lignified substances, Brauns (26) has noted that the extraneous materials of the starting wood must be pre-extracted as completely as possible, because they might not only be isolated as an inseparable part of the lignin but also might form condensation products with the lignin during the isolation procedure. It should be noted, however, that the starting wood has never been pre-extracted in some cases, such as studies on the chemistry of lignins isolated from the spent pulping liquors.

### 2.4.1 Native lignins

In 1939, Brauns (25) reported that a few per cent of the lignin of black spruce is found among the extractives obtained by extraction with aqueous ethanol and can be purified by series of precipitations with water and ether. The resulting cream-colored powder was found to possess all of the chemical properties associated with the total lignin and thus was termed Brauns Native Lignin (BNL). On account of its low

yield, however, it may be doubled whether BNL can be considered as representative for the bulk of the lignin in all respects (21).

In 1951, Nord and Schubert (123) tried to set the lignins of hardwood and softwood free for extraction with neutral solvents by removal of carbohydrates by biochemical decomposition. They utilized the "brown-rotting fungi", one of two main types of fungi which decompose the components of wood, to digest polysaccharides leaving lignin more accessible to solvent extraction. Enzymatically liberated lignin and BNL are outstandingly similar, as proven by extensive studies by Nord and his co-workers (122,123,152).

A few years later, Björkman (21) reported investigations on milled-wood lignin (MLW), isolated from spruce by using a vibrating ball mill in the presence of a non-swelling solvent, such as toluene. Björkman's method is based on the finding that about 30% of the lignin becomes extractable with dioxane-water, if wood is suspended in toluene and finely disintegrated in a vibratory ball mill (21). A conventional rotary ball mill was introduced by Brownell (31) to overcome some disadvantages, such as poor yields and length of time required for Björkman's procedure. According to his method, the milled wood is completely soluble in an aqueous solution of sodium thiocyanate, and the lignin is liberated by various treatments, such as transfer into the organic phase by liquid-liquid partitioning (31).

In 1979, Wegener and Fengel (167) used ultrasonics to speed up the dioxane extraction of ball-milled wood in their electron microscopic studies of lignin-polysaccharide complexes. By using a modified Björkman's

procedure with shaking and ultrasonic extraction, their lignin isolation method supplied highly reproducible yields of well defined lignins in a reasonably short time.

However, the best lignin preparation now available is probably the cellulolytic enzyme lignin, developed by Chang et al. (35). They treated wood meal which had been milled under toluene with an enzyme preparation possessing high cellulolytic and hemicellulolytic activities, and the lignin was isolated by extracting the digested material successively with aqueous dioxane.

#### 2.4.2 Lignins from industrial pulping processes

Lignins obtained from industrial pulping processes are always heterogeneous in nature. In all pulping processes the lignin is obtained in aqueous solution along with spent cooking chemicals and other materials dissolved from the wood. These lignins are usually not suitable for fundamental studies because of the presence of extractives in the original wood chips. Ready availability of the lignins from industrial pulping, however, caused these lignins to be used widely as experimental lignins, even without any purification (131).

Acidification of any of the commercial black liquors from the alkaline pulping, both kraft and soda processes, yields an alkali lignin. Isolation of alkali lignins, especially kraft lignins, was thoroughly reviewed by Pearl and his co-workers in a series of annual reviews (132).

In 1962, Merewether (117) investigated the precipitation of lignin from commercial *Eucalyptus* kraft black liquors with acids and reported on the optimum conditions necessary for satisfactory isolation. He also studied kraft black liquors prepared in the laboratory from extractive-free wood (117,118). The kraft lignins differ from lignosulfonates in that they are soluble only in alkaline solution above a pH of approximately 9.

The insolubility of kraft lignins in acidic solution has been overcome by Westvaco Corporation at Charleston, South Carolina (88) and a commercial lignin "Indulin" has been produced in three grades;

i) Indulin C (crude sodium salt of lignin), ii) Indulin B (purified sodium salt of lignin) and iii) Indulin AT (acidified lignin).

In 1980, Lundquist and Kirk (109) reported a simple purification procedure of an industrial kraft lignin, Indulin ATR-C by fractionation through a series of liquid-liquid extractions. A fraction which is water-insoluble, chloroform-soluble and ether-soluble is considered to be the purified kraft lignin and it accounts for more than 60% of the starting Indulin ATR-C.

The spent liquors from sulfite pulping processes contain more than 50% lignin in the form of lignosulfonic acids, which are mixed with sugars and other carbohydrate decomposition products, wood extractives, and pulping chemicals (131). Lignosulfonates have been isolated from spent sulfite liquors by a variety of means. Most of the procedures fall within a few general classes, including precipitation as an insoluble basic lignosulfonate, salting out with acids or salts, precipitation with alcohols and ion exchange (132,133).

The most important way of isolating and purifying lignosulfonates is the Howard process inaugurated by Marathon Corporation at Rothschild, Wisconsin (132). After removal of most of the sulfite and sulfate by lime addition to pH 10.5, more lime is added to the filtrate to give a basic calcium lignosulfonate which precipitates in the pH interval of 10.5 - 12.2. More than half the lignosulfonates quantity can be recovered by this process (84).

#### 2.4.3 Lignins from organosolv pulping

Organosolv cooking of wood in an aqueous organic solvent system with a proper catalyst at an elevated temperature provides an excellent procedure for simultaneous dissolution and almost quantitative recovery of both sugar and lignin fractions of wood. Organosolv pulping may be the only procedure which yields lignin as a by-product of pulping process in a less condensed state and free of inorganic contaminants(154).

Since Klason used 5% HCl-ethanol to extract lignin from spruce sawdust in 1893, many investigators have reported on a wide variety of organosolv lignins. In 1978 Paszner (129) reviewed a large number of papers on organosolv pulping. Among the organic solvents most frequently used are lower aliphatic alcohols, such as ethanol and butanol, ethylene glycol, glycerol, dimethyl sulphoxide and dioxane.

In an earlier study of various organic solvents that exhibit a certain degree of solvent action on the isolated lignins, Schuerch (97,153) showed that the ability of solvents to dissolve or swell the

isolated lignins increases as the hydrogen-bonding capacities of solvents increase and as their solubility-parameters ( $\delta$ ) approach a value of around 11. Although very powerful lignin-solvents, ketones, such as acetone ( $\delta = 10$ ), have not been used as often as lower aliphatic alcohols (129).

As early as 1931, Kleinert and Tayenthal (96) introduced aqueous ethanol solution with hydrochloric acid as a catalyst to cook wood above 150°C and obtained good yield of cellulose of low lignin content. In 1936, Aronovsky and Gortner (10) carried out a series of cooking on aspen sawdust and chips at constant pressure and in the temperature range of 160°C to 185°C with aqueous solution (1:1 ratio) of various organic solvents such as methanol, ethanol, propanols (n-, iso-), butanols (n-, iso-, tert-), amyl alcohols (n-, iso-, tert-), dioxane and ethylene glycol as cooking agents. They found that the normal primary alcohols were better pulping agents than the secondary or tertiary alcohols, and n-butanol yielded better pulp than was obtained with other solvents. However, when the same procedure was applied to pine, the result of delignification was poor and no pulp was produced (149). It was later found that delignification of hardwoods was about twice as fast as that of the softwoods when cooks of spruce and poplar sawdust were compared (94).

In a series of studies (93,94,95), Kleinert investigated the kinetics of bulk delignification that apply generally to organosolv pulping using aqueous ethanol solutions. It was found that delignification proceeded in two stages, an initial fast bulk delignification followed by a slow removal of the remaining lignin. Kleinert also demonstrated

that aqueous solutions of ethanol were better delignifying agents than ethanol alone (93,94,95). . . The preferred pulping agents were mixture of ethanol and water in the range between 20 to 75% ethanol by weight.

Kleinert also studied the influence of pH changes on organosolv pulping and reported that organic acids liberated in the pulping process had an accelerating effect upon delignification (94).

In 1973, Kůsíková and Polčín reported on the influence of varying concentrations of added acid catalyst and water content in the cooking liquor on the yield of extracted lignin by aqueous solutions of dioxane (97). It was found that pure dioxane was able to dissolve only very small amounts of lignin from wood. Addition of acid catalyst such as HCl increased the rate of delignification significantly (97, 153). The isolation of lignin with dioxane is basically an acidolytic splitting of the lignin macromolecule and a lignin-carbohydrate complex into individual components which are soluble in dioxane. In previous investigations on wood acidolysis, great importance was attached to the presence of a polar-solvent, mainly water, which substantially improved yields of the isolated lignin (121,135,153).

The potential of recovering lignins from large scale organosolv pulping process is very promising because of its many desirable properties. Among these, its high solubility in the usual lignin solvents such as ethanol, methanol, pyridine, chloroform, THF and acetone is most important. The isolated organosolv lignin retains its good solubility in lignin solvents-even after repeated precipitation and isolation from the spent liquor (37,39).

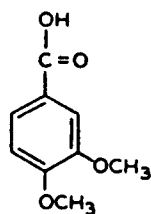
## 2.5 Characterization of Lignin

### 2.5.1 Degradation of lignin

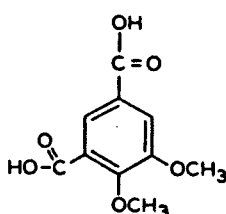
Direct proofs of structure of native lignin have been very few because the polymeric lignin involves many complex linkages. Nevertheless, the characterization of lignin has evolved from degradation and synthetic investigations.

#### 2.5.1.1 Strong oxidation

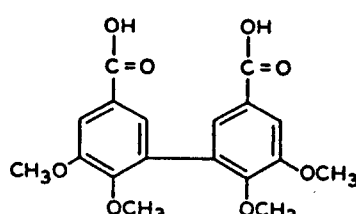
Strong oxidative degradation of methylated spruce lignin with permanganate (68) yields methoxyl-substituted aromatic acids, veratric acid (XIII), isohemipinic acid (XIV) and dehydro-diveratric acid (XV). The formation of isohemipinic acid (XIV) seemed to support the occurrence of  $\alpha$ -5 or  $\beta$ -5 condensed structures and that of veratric acid (XIII) indicated that noncyclic ether bridge between a side chain hydroxyl group and phenolic hydroxyl group of the adjacent unit were also important.



(XIII)



(XIV)



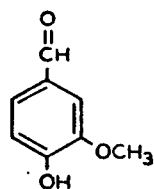
(XV)



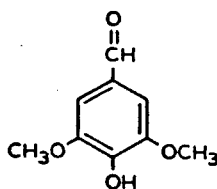
Though the oxidation of lignin with permanganate after methylation led to many interesting suggestions about the possible structure of lignin, the oxidation products failed to provide information concerning arrangement of side chains (144).

#### 2.5.1.2 Mild oxidation

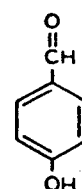
Mild oxidative degradation method such as nitrobenzene in the presence of hot alkali produced substantial yields of aromatic aldehydes (20,136,159). Spruce wood gave about 25% vanillin (XVI) based on the Klason lignin content, whereas mixtures of vanillin and syringaldehyde (XVII) were obtained from hardwoods. In addition to these two aldehydes, grasses afforded p-hydroxybenzaldehyde (XVIII). These degradation reactions giving three major aldehydes became one of the most important tools for the investigation of lignin materials. Later, small amounts of p-hydroxybenzaldehyde (XVIII) were also found in the oxidation mixtures from both softwoods and hardwoods (2).



(XVI)



(XVII)



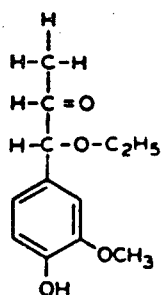
(XVIII)

In 1951, Stone and Blundell (159) published a simple procedure for the rapid microdetermination of aldehydes found in the nitrobenzene oxidation of lignified materials. This method involved separating the aldehydes chromatographically on a paper strip and thereby has become a valuable tool in differentiating between lignified and non-lignified materials.

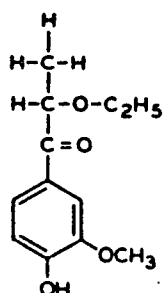
### 2.5.1.3 Ethanolysis

Solvolysis methods applied to lignin yield derivatives of phenylpropane (I). Acid catalyzed ethanolysis of coniferous wood lignin produces Hibbert's ketones (7,46,119,134) and is considered to be one of the mildest methods to isolate arylpropane monomers from lignin (100). A great many investigators applied this ethanolysis technique to a variety of materials as quantitative and qualitative analytical methods.

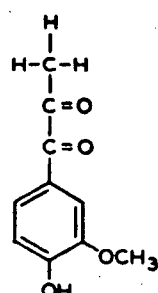
Hibbert and his co-workers (27,46,119) succeeded in isolating several monomeric phenylpropane units, so-called Hibbert's ketones (XIX, XX, XXI, XXII), from spruce wood by refluxing with 2-3% ethanolic hydrochloric acid.



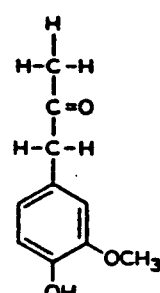
(XIX)



(XX)

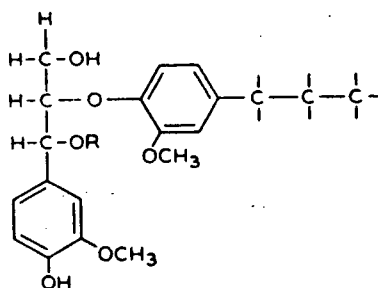


(XXI)



(XXII)

The importance of guaiacylglycerol- $\beta$ -aryl ether (XXIII), from which the phenolic Hibbert's monomers originated during ethanolysis, has been recognized by numerous researchers.



(XXIII)

In 1952, Alder and his co-workers (5) synthesized dimeric phenylpropane compounds of  $\beta$ -aryl ether which was considered to be incorporated in the lignin macromolecule either as end group or as an easily hydrolysable unit. They showed that Hibbert's ketones were formed through splitting of benzyl ether and  $\beta$ -aryl ether bonds of lignin.

According to Gardner (70), Hibbert's ketones (XIX, XX, XXI, XXII) were derived from ethanolysis of a ketol, 3-hydroxy-1-(4-hydroxy-3-methoxyl)-2-propanone (XXIV) via its enol (XXV).

The side-chain structures of these Hibbert's ketones certainly had to be regarded as modifications of the original structures caused by the acid during ethanolysis (2). Thus, the exact nature of C<sub>3</sub> side-chains in lignin is still an open question.

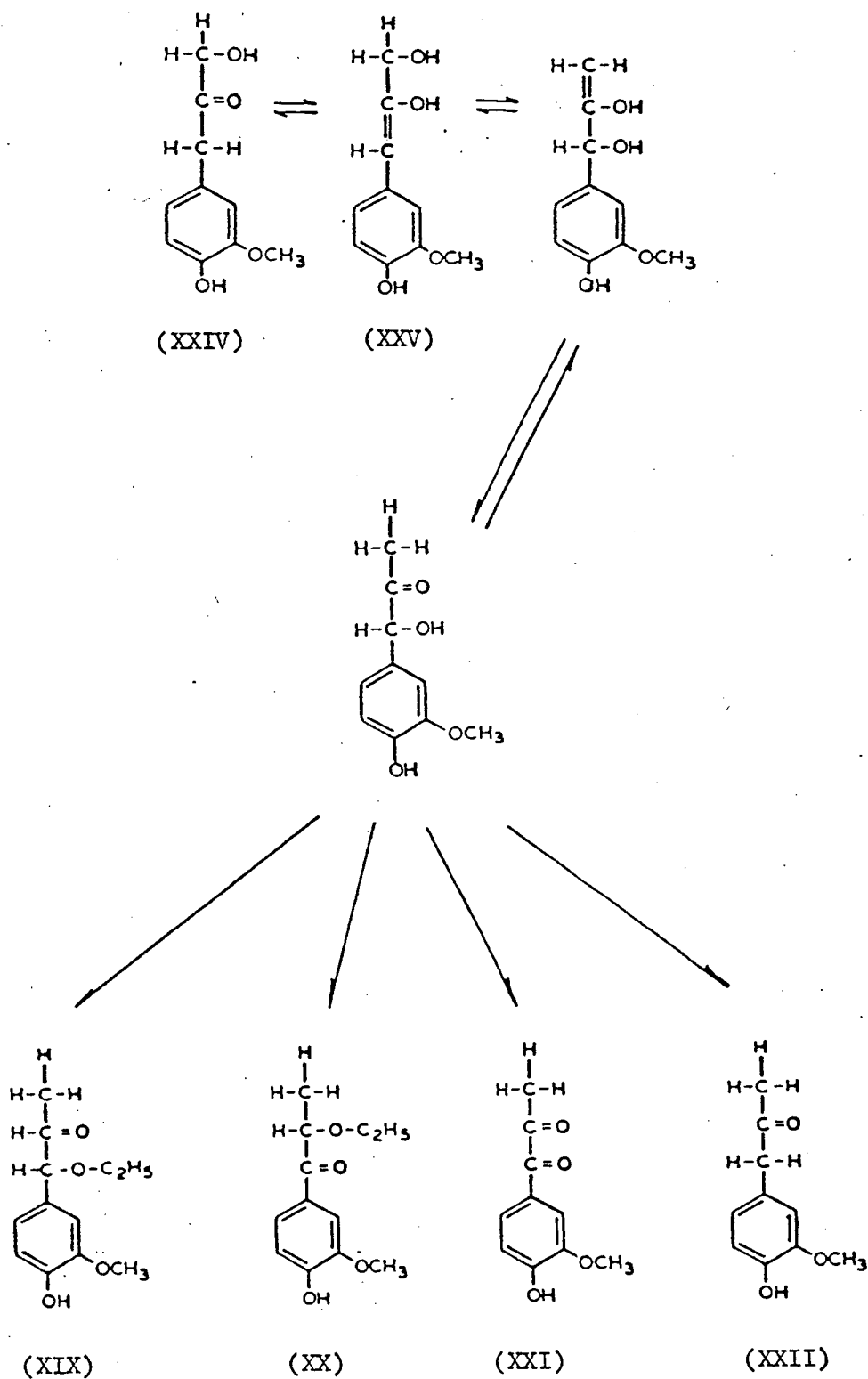


Figure 6. Formation of Hibbert's ketones (70).

#### 2.5.1.4 Hydrogenolysis

Together, mild hydrolysis and catalytic hydrogenolysis products from lignin represent almost all the linkage patterns which exist in the enzymatic dehydrogenation products of coniferyl alcohol. Catalytic hydrogenolysis mainly cleaves ether linkages and reduces in part the hydroxyls on side-chains (1,9,14,83,85,125,155).

In 1938, Harris and his co-workers (85) hydrogenated aspen methanol lignin in dioxane under high temperature and high pressure of hydrogen over a copper-chromatic catalyst obtaining fair yields of monomeric propylcyclohexane derivatives and it was established that lignin might be built up from  $C_9(C_6 - C_3)$  units. This experiment also constituted the first positive proof that lignin was predominantly aromatic in character.

Recently, dimers and trimers were isolated from hydrogenolysis products of protolignin and their structures were identified by means of ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy (114,115,116, 126).

#### 2.5.2 Spectroscopic studies on functional groups

Most of the earlier studies on UV spectra, IR spectra and NMR spectra of lignin, for determination of functional groups and linkages in lignin preparations, have been reviewed in detail by Goldschmid (76), Hergert (86) and Ludwig (105), respectively.

### 2.5.2.1 Benzyl alcohol and benzyl ether groups

Since Holmber, in the middle of 1930's, made the important suggestion that characteristic reactions of lignin were reactions of benzyl alcohol or benzyl ether, numerous investigations by UV spectra (5,22), IR spectra (56,67) and NMR spectra (120) have lent support this suggestion. The total amount of benzyl alcohol and benzyl ether per 100 C<sub>9</sub>-units of spruce lignin was estimated 24 groups (2,67). Benzyl alcohol and benzyl ether groups are known to be highly unstable under acid or alkaline conditions and therefore, they are almost likely absent in most pulp lignins (120).

### 2.5.2.2 Phenolic hydroxyl group

In the middle of 1950's Aulin-Erdtman (11,12) and Goldschmid (77,111) carried out series of studies independently to determine phenolic hydroxyl content of lignin. IR spectral analyses by Alder and his co-workers (2,4) showed that phenolic hydroxyl groups of the lignin units are largely etherified (  $\alpha$ -aryl ether or  $\beta$ -aryl ether structure) and determination of the amount of free phenolic hydroxyl groups should give a measure of the number of ether groups present. They reported that the amount of C<sub>9</sub>-units with free phenolic hydroxyl group in unaltered spruce lignin is less than 20 per 100

C<sub>9</sub>-units which means in a great majority of the guaiacylpropane units (II) the phenolic hydroxyl group is etherified (4).

#### 2.5.2.3 Methoxyl group

In 1967, Chang et al. (147) showed that the 280 nm UV absorption maximum of reduced softwood and hardwood lignins correlates well with the values of methoxyl groups vs. carbon ratio. They also reported that the calibration curves obtained from the ratio of the IR absorbance of the individual maxima from 1,600 cm<sup>-1</sup> to 1,045 cm<sup>-1</sup> and that of the maximum at 1,500 cm<sup>-1</sup> can be used to determine the corresponding methoxyl group per C<sub>9</sub>-unit of the lignin. More recently Faix and Schweer (57) determined the methoxyl content per C<sub>9</sub>-unit from the integrated NMR spectra of the acetylated lignin polymer models. Their results showed that the calculation by NMR spectra gives a little higher values than those obtained by the conventional methods.

#### 2.5.2.4 Carbonyl group

Studies on the IR spectra of various lignins indicated the presence of minor amounts of conjugated as well as non-conjugated carbonyl groups (6,35). Total number of carbonyl group is known to be 20 per 100 C<sub>9</sub>-units, of which half was found to be conjugated carbonyl groups (2,6).

### 2.5.3 Macromolecular properties of lignin

#### 2.5.3.1 Molecular weight distribution of lignin

The molecular weight of lignin and its distribution is one of the most fundamental characteristics of lignin. The determination of molecular weight of lignin macromolecules has been reviewed in detail by Brauns (26) and Goring (81).

In a series of studies (15,16,17) Benko characterized lignosulfonates by the diffusion coefficient method. He reported molecular weights of fractions obtained from a variety of lignosulfonates and calculated a molecular weight distribution curve from optical density readings of the diffusate. He also found that viscosity measurements on identical lignin samples in different solvents showed changes in molecular weight values due to interaction of the dissolved lignosulfonates with the solvent (15).

Marton and Marton (113), using a vapor pressure osmometer, obtained highly consistent number average molecular weights ( $\bar{M}_n$ ) of several kraft lignins. The  $\bar{M}_n$  values they obtained, however, ranged from 900 to 2,500 and gave only a one-sided picture of the polydispersity of lignin. Therefore, its use for macromolecular characterization of lignin is of limited importance.

In 1970, Brownell (30) measured the intrinsic viscosities and  $\bar{M}_n$  values of fractionated milled wood lignin. The results obtained suggested that the degree of branching was greater in high molecular



weight (5,000 - 19,000) than in low molecular weight (ca. 3,500) lignin fractions. Because of the non-linear structure of lignin, the intrinsic viscosity depends not only on the molecular weight but also on the degree of cross-linking and the interaction of electrostatic charges on molecular chains (82,139). Viscosity measurements were, therefore, not very valuable in molecular weight measurements of lignin solutions.

Goring and his co-workers (110), using the ultracentrifuge method, determined weight average molecular weight ( $\bar{M}_w$ ) of kraft lignins prepared from spruce sawdust. They obtained  $\bar{M}_w$  ranging from 1,800 to 51,000. The disadvantage of the ultracentrifuge method is that the polydispersity of lignin solution affects the sedimentation speed and thus the molecular weight results. Another difficulty is the **intense color of lignin solutions, because the concentration gradients developed in the ultracentrifuge cell are usually detected optically** (81).

Currently, the most rapidly developing method is gel permeation chromatography (GPC). Since its discovery in 1959, GPC has gained rapidly in success because the molecular weight distribution (MWD) can be determined quickly and easily. Depending on their size, the lignin macromolecules can diffuse in varying proportions into the porous volume of the column. Thus the elution volume of any particular fraction is a function of the dimension of lignin macromolecules and the size of the pores in the gel (81).

A great many GPC investigations on lignin have used dextran gels (Sephadex) (41,42,110,124,151,166,168) or agarose gels (Sephacrose) (89,90) as the stationary phase for GPC to determine molecular weights and MWD of lignin. Both types of gels (Sephadex gels and Sepharose gels) have certain disadvantages. The Sephadex gels can be used only up to molecular weight of 100,000 (89) and Sepharose gels up to  $4 \times 10^6$  (90). The latter contains charged groups which may interfere with the lignin (89). Although the cross linked copolymer of styrene and divinylbenzene beads (Styragel) is the most commonly used column packing gel for high polymers (127), no application of this gel for lignin macromolecules has been reported yet.

#### 2.5.3.2 Shape and size of lignin molecules

There are only a few papers describing lignin investigations by electron microscopy, mostly connected with degradation of the cell wall or with the investigation of polysaccharides containing a certain amount of lignin (57,58,60,61,62,99).

In 1963 Rezanowich et al. (139) reported that the molecules of dioxane lignin had spherical configuration in solution. The spherical shape was also supported by agreement between the sedimentation equilibrium molecular weights and values obtained by substitution of intrinsic viscosity and diffusion constant into an equation derived from the Einstein viscosity relationship for spherical particles. The low intrinsic viscosity of lignin solutions suggested that lignin molecules behave like Einstein spheres in solution (81).

Further evidence for the spherical shape of lignin macromolecules was provided by electron micrographs of high molecular weight fractions of sodium lignosulphonates (138). Alkali lignins and organosolv lignins were found to behave more like Einstein spheres than the lignosulfonates (81). In 1978 Křsíková *et al.* (99) confirmed that BNL and methanol lignin from beech wood and MWL from spruce wood showed characteristic spherical aggregates of lignin macromolecules. In their electron microscopic investigations on the above lignin samples, they found that all these lignins had characteristic structures of small spherical particles of about 100 to 400 nm. A statistical particle size distribution was reported for Björkman lignin and BNL by Fengel (59). Further, his studies indicated little, if any, effect of the isolation method on granular shape and size distribution of the above lignins.

In summary, it is evident that most isolated lignins exist as high molecular weight fractions and show behavior in solution characteristic of Einstein spheres of microscopic to macroscopic size. The inside structure of such spheres has not been investigated yet.

### 3. MATERIALS AND METHODS

The present investigation involved the isolation and limited chemical and physical characterization of the organosolv lignins from extractive-free Douglas-fir sawdust. Dissolved sugars were not analyzed in this study. Nor were the pulps analyzed beyond their yield and residual lignin content.

Though Douglas-fir was exclusively used as the starting material for this thesis, other species were also investigated during the preliminary cooking experiments for comparative purpose and included spruce, aspen, birch, sugar cane and wheat straw (39).

The fractionation of the lignins from organosolv cooking was done according to the scheme summarized in Fig. 7. Difficulties were experienced with quantitative isolation of the water-soluble fraction.

For characterization of the isolated lignins, some of the most powerful tools available for lignin investigations, such as high-speed GPC, scanning electron microscopy (SEM) as well as UV, IR, and NMR spectrometries were employed.

#### 3.1 Materials

##### 3.1.1 Selection of starting material

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.) sawdust served as starting material for this study to isolate and characterize the lignins from organosolv cooking. Douglas-fir sawdust comprises a substantial portion of the raw material supply for many pulp mills in

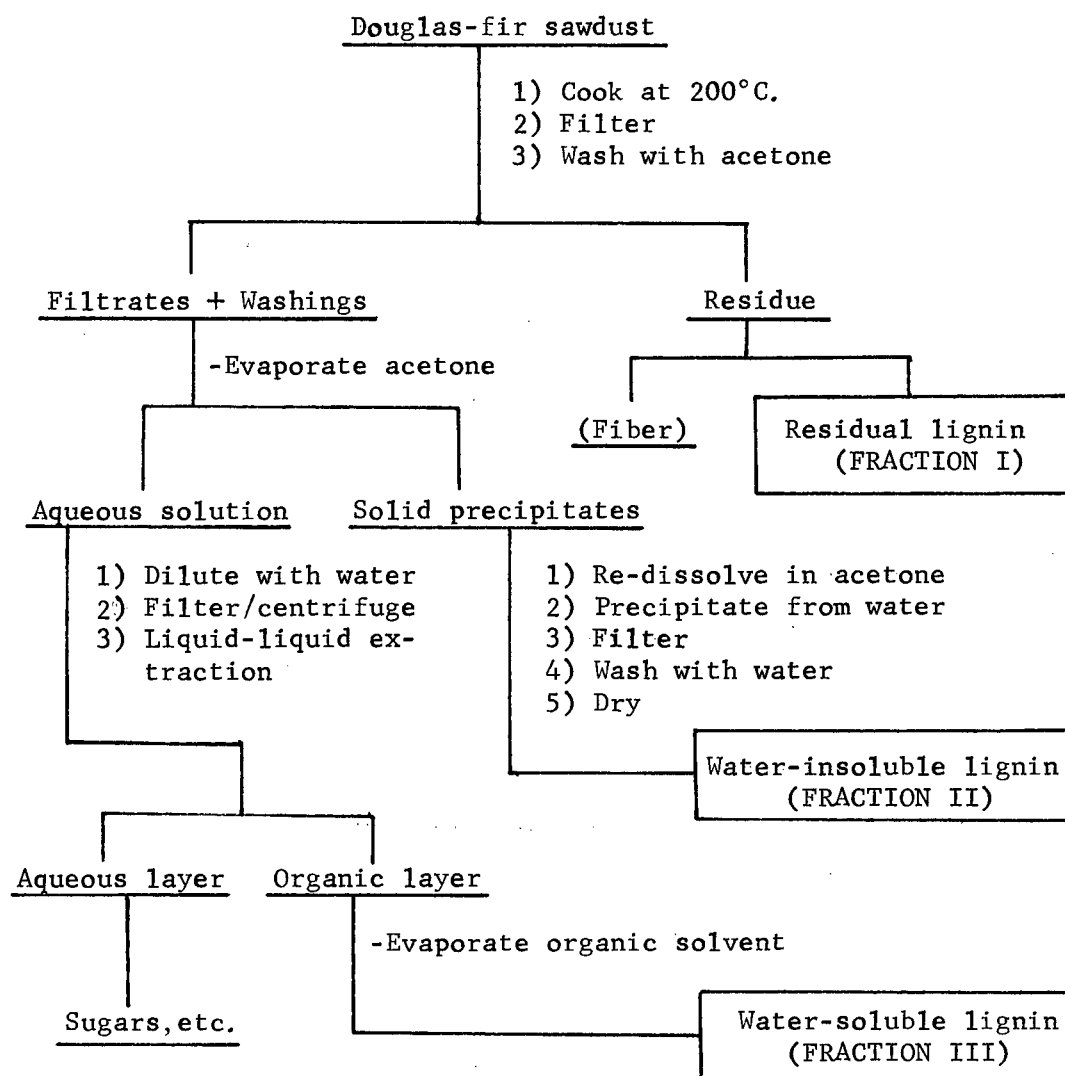


Figure 7. Fractionation of lignin from organosolv cooking.

in the Pacific Northwest (34) and is readily available in large quantities. The fresh sawdust was obtained from the production line of L & K Lumber, Ltd., North Vancouver, British Columbia. The Douglas-fir trees were about 80 years old and originated from the Pacific coastal region.

The particle size of the sawdust (sp. gr.=0.42) selected for this study covered a wide range. The results of a sieve analysis on particle size distribution of the air-dry Douglas-fir sawdust sample are shown in Table 1.

Table 1. Sieve analysis of Douglas-fir sawdust.

Sieve size(mesh)	<10	10-20	20-40	40-60	60-80	>80	Total
Frequency(%)	30.9	36.7	21.9	6.3	1.6	2.6	100

The fraction accepted for this study was that passed through a 10-mesh sieve and retained on a 40-mesh sieve, which constituted about 60% (based on air-dry weight) of the fresh sawdust collected.

### 3.1.2 Preparation of extractive-free sawdust samples

In order to obtain extractive-free sawdust as cooking material, the air-dry sawdust sample was extracted with a mixture of 95% ethanol and benzene (1:2 by volume) in a large Soxhlet extractor for 8 hours followed by extraction with 95% ethanol for 40 hours (a modified procedure of TAPPI Standard T12m-59). The content of alcohol-benzene extractives was found

to be 3.9% of oven-dry un-extracted sawdust.

After proper washing with ether and air-drying, the extractive-free sawdust sample was stored in the CTH room (21°C/50% RH) before moisture content was determined. The moisture content of the extractive-free sawdust was 9.44%. Analysis of chemical composition of the extracted sawdust was also carried out, and the results are given in Table 2.

### 3.1.3 Preparation of organosolv lignin samples

A 5 g (oven-dry basis) portion of the extractive-free sawdust sample was placed in a 65 ml capacity stainless steel bomb-digester along with 50 g of cooking liquor (wood/liquor ratio=1:10). The cooking liquor consisted of acetone and water (60:40 by volume), with various concentrations of hydrochloric acid as the catalyst.

Cooking was carried out in a glycerine-bath equipped with Universal Relay (Type R-10), PTR-Electronic Controller (Type R-20/2) and P-120 Electronic Programmer, for the desired periods of time at 200°C. Each cook was duplicated to obtain replicate yields of pulp and lignin fractions.

After cooking, the undissolved lignocellulosic residue was separated from the spent liquor by vacuum filtration and washed with acetone (ca. 100 ml). The residue was slushed with acetone (ca. 300 ml) in a blender at a low speed for further disintegration to remove the trapped lignin. The slurry was filtered and washed with fresh acetone (ca. 200 ml).

The residual fibers were then dried in an oven at 105±3°C and pulp yield and the residual lignin (FRACTION I) were determined.

The combined solution of filtrates and washings was evaporated on a flash evaporator at 50°C to obtain dark brown mass (a quasi-molten phase) of crude lignin and a clear yellowish aqueous solution which contained sugars and water-soluble lignin (FRACTION III).

The dark brown lignin mass was redissolved in a minimum amount of acetone (ca. 20 ml) and precipitated into an excess amount of distilled water (1,000 ml) with vigorous stirring. The water-insoluble lignin precipitates were collected by vacuum filtration and washed thoroughly with warm (40-45°C) water. This powdered water-insoluble organosolv lignin (FRACTION II) was dried over phosphoric anhydride in a desiccator placed in an oven at 50°C.

#### 3.1.4 Preparation of acetylated lignin samples

Acetylation of the isolated organosolv lignin samples was done by the method used by DeStevens and Nord (48).

The lignin sample (0.4 g) was dissolved in pyridine (6 ml), and acetic anhydride (5 ml) was added to the solution with stirring. The mixture was then allowed to stand for 48 hours at room temperature and centrifuged at a rotor-speed of 12,000 rpm for 15 minutes. The clear solution portion was separated from the fine precipitates and poured into ice-water (200 g) to precipitate the acetylated lignin. The precipitates were collected by vacuum filtration through a Millipore filter (pore size: 0.2  $\mu$ m) and washed with 0.1 N hydrochloric acid (100 ml) to neutralize any remaining pyridine. The acetylated lignin was then washed with distilled water several times until the filtrate was no more acidic and dried over phosphoric anhydride at 50°C as mentioned above.



### 3.1.5 Preparation of reduced lignin samples

Borohydride-reduced lignin samples were prepared by a modified method of the procedures adapted by Alder et al. (4) and Gierer et al. (72).

The isolated lignin (0.12 g) was dissolved in a mixture of ethanol (8 ml) and 0.1 N sodium hydroxide (2 ml) under nitrogen atmosphere. Sodium borohydride (0.04 g) and additional water (4 ml) were added to the mixture with stirring. The reaction mixture was allowed to stand overnight and acidified to pH 2 with dilute hydrochloric acid. The precipitates formed were collected by centrifuging and washed with water several times. The reduced lignin was then dried over phosphoric anhydride at 50°C.

## 3.2 Methods

### 3.2.1 Analysis of lignin fractions

#### 3.2.1.1 Klason lignin

To determine acid-insoluble Klason lignin content of the extractive-free sawdust, the procedure described in TAPPI Standards T13 os-54 was followed. A modified secondary hydrolysis with 3% sulfuric acid was used by treating the reaction mixture in an autoclave under pressure of 20 psig steam pressure and 127.5°C for 1 hour.

The insoluble residue (Klason lignin) was collected by vacuum filtration on a medium porosity glass crucible, dried at 105 ± 3°C and weighed. The filtrate from the filtration was saved for the

determination of acid-soluble lignin content.

### 3.2.1.2 Acid-soluble lignin

The acid-soluble lignin was determined according to TAPPI Useful Method 250. The acid solution, which contained the acid-soluble lignin, was obtained from the Klason lignin determination.

The maximum UV absorbance of the acid solution was measured around 205 nm and used for calculation of acid-soluble lignin content by using the following equation (TAPPI UM 250). A Unicam SP 800 Spectrophotometer was used to obtain the UV spectrum.

$$\text{Lignin, \%} = \frac{B \times V \times 100}{1000 \times W}$$

where V = total volume of solution (ml)

W = oven-dry weight of wood (g)

B = lignin content (g/1000ml) and

B can be calculated by:

$$B = \frac{A \times D}{110}$$

where A = UV absorbance

D = dilution factor

### 3.2.1.3 Residual lignin

The residual lignin content (FRACTION I) in the fiber residue was determined by the micro Kappa number method described in TAPPI Useful

Method 246. Sample preparation was done according to TAPPI Standards T 236 m-76. The residual lignin in the pulp was computed from the following equation (32):

$$\text{Residual lignin, \%} = \text{Kappa number} \times 0.15$$

#### 3.2.1.4 Water-soluble lignin and degradation products

The aqueous portion, following evaporation of the organic solvent of the cooking liquor, was separated and diluted to 100 ml with distilled water. In order to separate the water-soluble lignin from the dissolved sugars, the yellowish aqueous solution was extracted with chloroform in a specially designed liquid-liquid extractor. The organic layer from the extraction was concentrated to about 10 ml on a rotary evaporator at  $50 \pm 5^\circ\text{C}$  and a thin-layer chromatography (TLC) sample was taken from the concentrated solution at this stage. The evaporation of the organic layer was continued until a highly viscous syrup-like residue (FRACTION III) was obtained.

A small portion (0.1-0.5 g) of the water-soluble lignin (FRACTION III), which was not readily soluble in neutral water or methanol, was re-dissolved in acetone (15 ml) and diluted to 50 ml with methanol. The clear solution was concentrated on a rotary evaporator at a low temperature ( $40^\circ\text{C}$ ) to remove the acetone. To remove any remaining acetone, a large amount (ca. 50 ml) of methanol was added to the sample and concentrated again. This procedure was repeated several times until no detectable acetone by UV absorption remained.

The acetone-free methanol solution was then diluted to the desired concentration with methanol and UV spectrum was taken on a Uni-

cam SP 800 Spectrophotometer. The calculation for the water-soluble lignin content was essentially the same as that suggested by TAPPI Useful Method 250, but methanol was used as reference instead of 3% sulfuric acid.

Qualitative TLC analysis was conducted by the methods described by Kratzl and Paszner (100) and Barton (13) with minor modifications, using TLC glass plates (20 cm x 20 cm) coated with 0.25 mm thick Silica Gel G. Benzene-acetone (60:40), methanol-chloroform (30:70), benzene-ethanol (150:22) and benzene-chloroform-methanol (70:28:2) were used as developing systems.

The plates were first examined under UV light and the spots were identified by their  $R_f$  values and colors developed upon spraying with the various reagents. The spraying reagents used were Folin-Denis reagent (100) and diazotized sulphanilic acid (13). To detect the presence of carbonyl groups and Hibbert's ketones, 2,4-dinitrophenyl hydrazine (80) and ferric chloride-potassium ferricyanide reagents (70) were also used.

### 3.2.2 Chemical analyses of isolated organosolv lignins

Some equipment, such as elemental analyzer, was not directly accessible for the present study. Due to the limited funds available for rental, a minimum number of acetylated or reduced organosolv lignin samples, representing series of two cooking variables (cooking time and acid catalyst concentration), were selected for the extensive chemical characterization of the isolated organosolv lignins.

For cooking time series, selected samples PC-11 (5 min),

PC-12 (9 min), PC-13 (12 min), PC-14 (17 min) and PC-15 (20 min), all prepared with the same concentration of acid catalyst (0.05 N HCl), and for acid concentration series, PC-9 (0.025 N HCl), PC-14 (0.05 N HCl) and PC-19 (0.1 N HCl), all of the same cooking period (17 min), were selected for elemental and spectral analyses.

These selected samples were part of the complete experimental scheme for both cooking time and acid concentration series, which will be presented later (Table 3) in connection with discussion of the effect of various cooking conditions on lignin yield.

### 3.2.2.1 Elemental analysis and methoxyl content determination

The elementary composition of the acetylated lignin was determined by standard methods of organic combustion analysis for per cent carbon and hydrogen contents based on the freeze-dry lignin samples.

A small amount (0.7 mg) of the freeze-dried acetylated lignin sample was weighed in a tin container loaded into the sample holder and injected into a combustion reactor at 1,010°C. The combustion gases were carried by a constant flow of helium through to the catalytic section of the reactor for complete oxidation to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{N}_2$  and  $\text{N}_x\text{O}_y$ . The gas mixture flowed into a second reactor kept at 644°C which was filled with copper for reduction of the nitrogen oxides. The gas mixture was then directed into a chromatographic column for  $\text{N}_2$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  separation. The gas components were quantitatively analyzed by a thermal conductivity detector. The machine used was an

Elemental Analyzer-Model 1106 equipped with Model CSI 38-Digital Integrator.

For the determination of the methoxyl content of the isolated lignin and extractive-free sawdust samples, TAPPI Standards T209 su-72 was followed with a few modifications. The test specimens (0.1 g for lignin; 0.3 g for sawdust) were reacted with 56.6% hydroiodic acid (6 ml) and propionic acid (2 ml) at 150°C for 40 minutes. The resultant methyl iodide was removed from the reaction flask by a current of nitrogen and oxidized in an acidic solution of potassium acetate containing bromine to give iodic acid.

The iodic acid was determined by titration with 0.1 N sodium thiosulfate. The methoxyl content was then calculated by the following equation (TAPPI T 209 su-72).

$$\text{Methoxyl, \%} = \frac{0.0517 (A - B)}{W}$$

where, A = volume of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution required for specimen (ml)

B = volume of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution required for blank (ml)

W = moisture-free weight of the specimen (g).

#### 3.2.2.2 Ultraviolet spectra

The UV spectra for the isolated lignin and the reduced lignin samples were recorded with a Unicam SP 800 Spectrophotometer. The

procedure selected for the determination of the phenolic hydroxyl group was the method of Goldschmid (77).

The isolated lignin sample (0.2 g) was dissolved with gentle heating (50-60°C) in pH 12 buffer solution (100 ml) which was made of 6.2 g of boric acid in 1,000 ml of 0.1 N sodium hydroxide. A portion of this lignin solution (2 ml) was diluted to 50 ml with pH 12 buffer solution (alkaline solution), and another portion (2 ml) was neutralized with 0.1 N sulfuric acid (2 ml) and diluted to 50 ml with pH 6 buffer solution (neutralized solution).

The differential spectrum was determined by measuring the absorbance of the alkaline solution relative to that of the neutralized solution which was placed in the reference cell of the spectrophotometer as the blank.

Phenolic hydroxyl groups were estimated by using the following equation (77).

$$\text{Phenolic hydroxyl, \%} = \Delta_{a,\text{max}} \times 17/41$$

where  $\Delta_{a,\text{max}}$  = absorptivity difference at maximum peak (1/g.cm).

#### 3.2.2.3 Infrared spectra

IR spectra of the borohydride-reduced lignin samples were obtained with a Perkin-Elmer 521 Spectrophotometer with an extended

range interchange which can eliminate the environmental problems, such as sensitivity to moisture and temperature. The frequency range was  $4,000\text{ cm}^{-1}$  to  $250\text{ cm}^{-1}$ , with accuracy of  $\pm 0.5\text{ cm}^{-1}$  and reproducibility of  $0.25\text{ cm}^{-1}$ .

The procedure followed was similar to the method adapted by Naveau (121). The KBr pellets were made by mixing the reduced lignin (4 mg) and potassium bromide (200 mg) and pressing under a pressure of 12,000 psi into a 1 cm diameter pellet.

#### 3.2.2.4 Nuclear magnetic resonance spectra

NMR spectra of the acetylated lignin samples were obtained on a Varian EM-390 90 MHz NMR Spectrometer.

The acetylated lignin (ca. 10 mg) was dissolved in deuteriochloroform ( $300\text{ }\mu\text{l}$ ) and filtered through glass wool into a 5 mm thin-wall sample tube. Tetramethylsilane (TMS) was added as an internal reference standard. Sweep width was 10 ppm and sweep time was 2 minutes. Spectrum amplitude varied from 5000 to 6000. The integration of the spectrum was recorded to obtain the relative peak areas.

#### 3.2.3 Macromolecular analyses of isolated organosolv lignins

##### 3.2.3.2 Gel permeation chromatography

GPC results were obtained on a high-speed GPC, Water Associates Model ALC/GPC-201 equipped with a differential refractive index detector.



The acetylated lignin sample (25 mg) was dissolved in tetrahydrofuran (5 ml) to make about 0.5% solution. To minimize the possibility of viscous fingering, the solution was filtered through two Millipore filters (pore size:  $0.45\ \mu\text{m}$ ).

The injection of the sample ( $250\ \mu\text{l}$ ) was done with the aid of a Model U6K universal injector. The separation of fractions with different molecular weights was accomplished through a series of four columns packed with different sizes of highly porous gel particles ( $\mu$ -Styragel). The columns used were  $10^4\text{\AA}$ ,  $10^3\text{\AA}$ ,  $500\text{\AA}$  and  $100\text{\AA}$  for molecular weights of 10,000–200,000, 1,000–20,000, 50–10,000 and 0–700, respectively. The pressure of the flowing solvent system was 1,000 psi and the flow rate was 1 ml per minute.

The differential refractometer detected a change in refractive index as small as  $10^{-7}$  RI units which corresponds to a concentration change of 1 ppm of lignin sample. An X-Y recorder converted the differential refractometer signal to a continuous trace on the chart. The time required for a complete run was about 50 minutes. To construct a calibration curve, the detector count number was plotted on the X-axis against the corresponding value of a known molecular weight on the logarithmic Y-axis on semi-log paper (Fig. 8).

To calculate the weight average molecular weight ( $\bar{M}_w$ ) and the number average molecular weight ( $\bar{M}_n$ ), peak height of each count number was measured and the polydispersity was expressed by the ratio of  $\bar{M}_w/\bar{M}_n$ .  $\bar{M}_w$  and  $\bar{M}_n$  were calculated by the following equations (8).

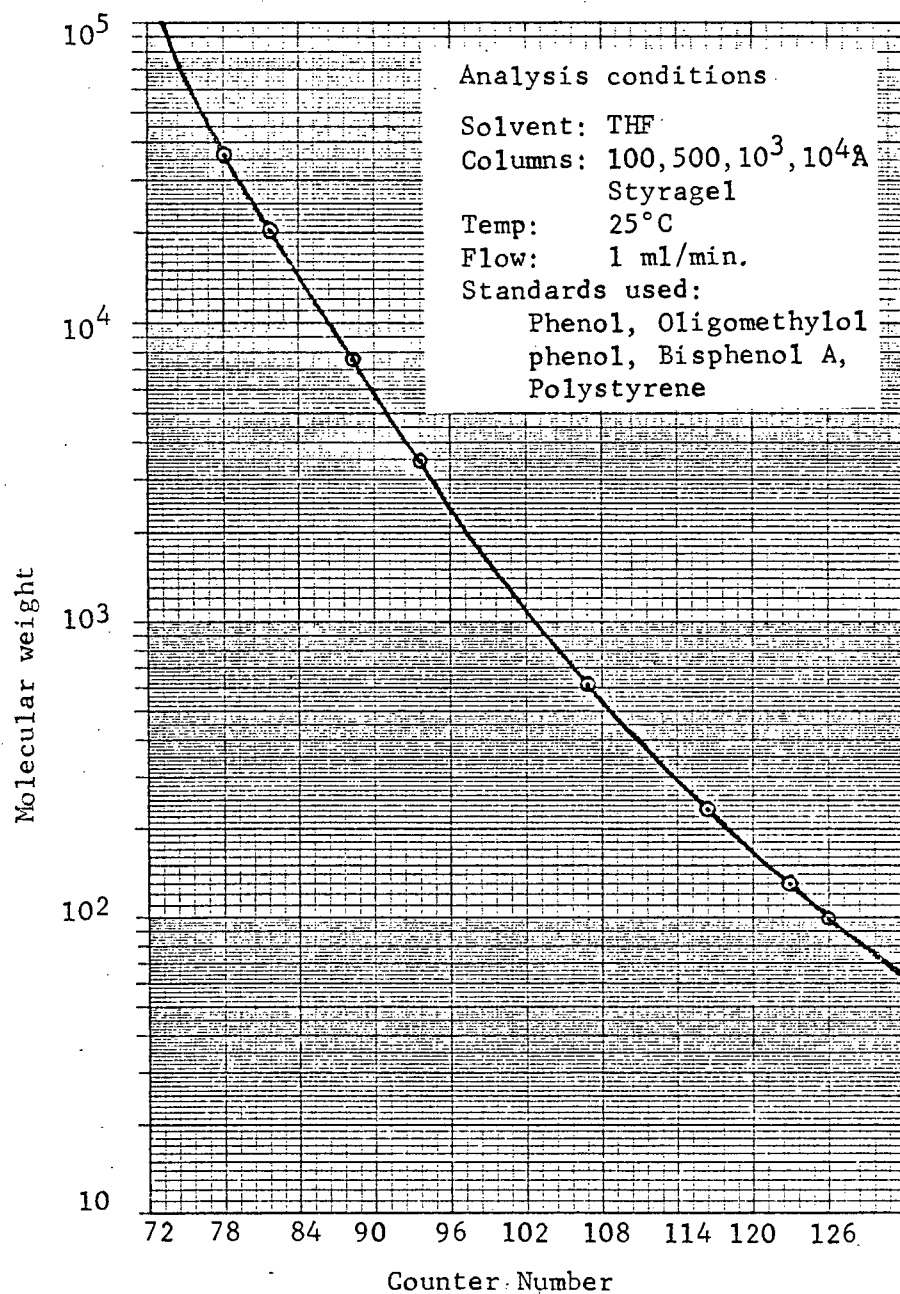


Figure 8. GPC calibration curve.

$$\bar{M}_w = \frac{\sum (H \times M)}{\sum (M)}$$

$$\bar{M}_n = \frac{\sum (H)}{\sum \left(\frac{H}{M}\right)}$$

where, H = height of peak of each count number

M = molecular weight converted from count number  
(from calibration curve, Fig. 8)

Two selected sample series, PC-11, 12, 13, 14 and 15 for cooking time and PC-9, 14 and 19 for acid concentration series, were investigated for GPC analysis of the isolated organosolv lignins.

### 3.2.3.2 Scanning electron microscopy

An ETEC Autoscan SEM was used to investigate the nature of the particles of the acetylated lignin samples. The primary beam voltage applied was 20 KV.

The SEM specimen was prepared by dissolving a small amount (0.01 g) of the acetylated lignin sample in acetone (1 ml) and the solution was added drop by drop to distilled water (ca. 8 ml) with vigorous stirring and diluted to 10 ml with distilled water.

One drop of the suspension was put on a Millipore filter and air-dried. In order to prevent electrostatic charging, a thin gold film was deposited on the surface of the specimen. The specimens thus prepared were observed by SEM and photographed with a Polaroid 545 Camera at 20,000 magnification.

The samples used for the SEM investigation were exactly the same as those used in the GPC analysis.

#### 4. RESULTS

The chemical composition of the extractive-free Douglas-fir sawdust is presented in Table 2. The contents of  $\alpha$ -cellulose, hemicellulose, Klason lignin and acid-soluble lignin were not corrected for ash content which is about 0.2% for Douglas-fir wood (141). Table 2 also includes methoxyl content of the extractive-free sawdust.

The yields of pulp and lignin fractions are tabulated in Table 3. The yields of pulp and the isolated organosolv lignin (FRACTION II) are further plotted against cooking time and acid concentration in Figs. 9 and 11, respectively. Fig. 10 shows temperature rise inside the bomb-digester, which was measured by copper-constantan thermocouple, during a prolonged period (30 min) of cooking. The results of preliminary cooking experiments with various wood species are presented in Table 4. Changes in pH value of the cooking liquors with various concentrations of acid catalyst, before and after cooking for 20 minutes, are compared in Table 5.

Table 6 indicates characteristic TLC colors and  $R_f$  values of the known degradation compounds and phenolic model substances which are most likely present in the spent cooking liquor. TLC results, obtained from PC-15 (0.05 N HCl; 20 min. cook) after removing the sugars from the spent liquor, are presented in Table 7.

Table 8 presents the elementary compositions of some selected acetylated lignin samples representing several cooking time and acid concentration series. Methoxyl contents of the selected acetylated lignin samples and their parent lignins are also given in the same table.

Fig. 12 demonstrates a typical NMR spectrum of the acetylated organosolv lignin from extractive-free Douglas-fir sawdust with description of each region of the spectrum. Assignments of the signal regions are listed in Table 9. Figs. 13-19 reproduce the NMR spectra of the selected acetylated lignin samples. Comparison of these NMR spectra is illustrated in Fig. 20. From each NMR spectrum, the relative intensities of various proton types were obtained and the results are computed in Table 10. From these values, contents of the various functional groups in lignin molecules were estimated and the results are expressed as the number of functional groups per  $C_9$ -unit of lignin molecule in Table 11.

Effects of cooking time and acid concentration on the IR spectra of the selected reduced organosolv lignin samples are compared in Figs. 21 and 22, respectively. Assignments of absorption bands in the IR spectra are tabulated in Table 12.

Fig. 23 presents the difference curve of UV absorption of the selected reduced lignin samples. From maximum peaks of the difference curve, the molar absorptivity was measured for each sample and the results along with the calculated phenolic contents are given in Table 13.

The values of  $\bar{M}_w$  and  $\bar{M}_n$  as well as the polydispersity indices obtained from GPC analysis are presented in Table 14. Effect of cooking conditions on  $\bar{M}_w$  and  $\bar{M}_n$  of the selected acetylated lignin samples are summarized in four diagrams in Fig. 24. Comparative molecular weight distributions, as affected by cooking time and acid catalyst concentration, are illustrated in Figs. 25 and 26, respectively.

Scanning electron photomicrographs depicting particle size variation of the uniformly precipitated lignins as affected by cooking time and acid concentration are shown in Figs. 27 and 28, respectively. Changes in particle size due to varying cooking time and concentration of acid catalyst are shown in Table 15 by tabulating particle size frequency distributions of the acetylated lignin samples. From the data, particle size distribution diagrams for the cooking time and acid concentration series were constructed and are presented in Figs. 29 and 30, respectively.

## 5. DISCUSSION

In the present study, the main objectives were to isolate the precipitable organosolv lignin from Douglas-fir sawdust and to characterize its chemical and macromolecular properties. For this reason, only brief treatment was given to other fractions, such as water-soluble lignin or degradation products of lignin, without making a serious effort in completing the picture.

No analysis was conducted to investigate the dissolved carbohydrates (hemicelluloses and glucose). Such sugar analysis was carried out earlier and the results were published (36, 37), and thus was not considered as part of the present study.

### 5.1 Chemical Composition of Extractive-free Douglas-fir Sawdust

As shown in Table 2, the average lignin content of the extractive-free Douglas-fir sawdust was found to be 31.81 %, based on the oven-dry extractive-free sawdust, or 30.66 %, based on the oven-dry unextracted sawdust. While most of the recorded lignin contents of Douglas-fir lie between 27-29 %, a substantial variation (24.5-33.5 %) in the content has also been reported (148). Such a variation exists not only between members of a single species grown under different environmental conditions or from different seed source, but also within incremental growth zones of a single tree (170). Since the sawdust used in the present study was obtained from an industrial lumber production line, it was not

Table 2. Chemical composition of extractive-free Douglas-fir sawdust.

Wood component	Amount (%) <sup>a</sup>	Test method used
Holocellulose	68.19 <sup>b</sup> (65.74) <sup>c</sup>	Acid chlorite method
$\alpha$ -cellulose	43.04(41.49)	TAPPI T203 os-61
Hemicellulose	25.15(24.25)	
Lignin	31.81 (30.66)	
Klason lignin	31.50(30.37)	TAPPI T13 os-54
Acid-soluble lignin	0.31(0.29)	TAPPI UM 250
Weight loss(Extractives)	- (3.60)	
Total	100(100)	
Methoxyl content	5.19(5.00)	TAPPI T209 su-72

<sup>a</sup>Not corrected for ash content.

<sup>b</sup>Percentage values based on oven-dry extractive-free sawdust.

<sup>c</sup>Percentage values in parentheses based on oven-dry unextracted sawdust.



possible to trace the natural origin of the relatively high lignin content. Klason lignin, which was 31.50 % of the extractive-free oven-dry sawdust, accounted for 99.06 % of total lignin, whereas the acid-soluble lignin fraction was less than 1 % of the total lignin content.

The contents of  $\alpha$ -cellulose and hemicellulose of the extractive-free sawdust were found to be 43.04 % and 25.15 %, respectively (Table 2). The cellulose content of softwoods was reported to be between 41-45 % (141, 160) and it is also well known that higher or lower values indicate the presence of reaction wood (160). Kennedy and Jaworsky (92) reported that no fully satisfactory explanation for a variation in cellulose content of Douglas-fir (70 to 85 years old) could be found in spite of thorough analyses on crown class, site, radial position, growth rate and per cent summerwood. However, it was suggested that most of the variation in cellulose content could be attributed to inherent genetic characteristics of individual trees. Considering the fact that the collected sawdust was a mixture of all possible cases, the result of  $\alpha$ -cellulose content (43.04 %), which is very close to the reported average value, is quite normal.

The weight loss of original sawdust due to alcohol-benzene extraction, which can be considered to be the content of extraneous substances extracted was found to be 3.60 % of the unextracted sawdust. This value is slightly lower than the recorded value of 4.4 % (141). The reason for this may be explained by the fact that extractive deposits inside lumens can not be completely extracted even with prolonged alcohol-benzene extraction (91).

The methoxyl content of the extractive-free sawdust was found to be 5.0%, which is well within the reported range of 4.97-5.67% for Douglas-fir wood (148,170).

## 5.2 Isolation of Organosolv Lignins

As mentioned earlier, no method by which protolignin can be isolated in an unchanged state has yet been developed. Isolated lignins from organosolv pulping or saccharification processes seem to be the only lignins which can be generated on a large scale, in a less-condensed state and free of organic or inorganic impurities (154).

It is known that isolation of lignin from wood by acid-catalyzed organosolv cooking is essentially an acid hydrolysis of polymeric lignin molecules and of the lignin-carbohydrate matrix (97, 121,169). The acidified organosolv cooking system is far more efficient in delignification and sugar hydrolysis than aqueous acid hydrolysis due to superior penetration power of the organic solvent and simultaneous dissolution of all hydrolysed products, including lignin. In order to find the optimum composition of the aqueous organic cooking liquor, not only the yield of lignins, but also solubility of the isolated lignins in various solvent systems were compared. The optimum composition of the cooking liquor was found to be an acetone-water system with a ratio of 3:2 by volume.

Acetone was chosen as the organic component of the aqueous organic solvent system because of its excellent solvent power for the polymeric lignin fragments. It is reported that the ability of solvents to dissolve an acidolytically attacked lignin macromolecule or lignin-carbohydrate complex increases as their solubility parameter

( $\delta$ ) approaches the value of around 11 (153). Acetone has a value of  $\delta=10$ , i.e. it is very close to the optimum value for lignin solubility. For the simultaneous removal of liberated lignin fractions during acid hydrolysis, a solvent having such high solubility parameter must be chosen (97,129,153).

After cooking, there are several choices available for isolation of the dissolved lignins from organosolv spent cooking liquor. While perhaps direct precipitation of the spent liquor into an excess (8 to 15 volumes) of distilled water is the easiest way, the recovered lignin fractions after such a procedure do not provide good yields for lignin mass balance because of the large amounts lost due to partial dissolution of low molecular weight lignin fractions in water.

Quantitative recovery of the water-insoluble fraction of lignin is best accomplished by evaporation of the organic solvent from the spent liquor on a flash evaporator at low temperature. This obtains a mass of crude lignin and slightly yellowish clear aqueous solution which contains dissolved sugars and the water-soluble lignin fraction. The concentration of dissolved sugars in the aqueous solution did not exceed 21% even from the prolonged cooks (20 min), and it was noted that all the dissolved sugars were present as monomers in the spent liquor (37). Water-soluble lignin (FRACTION III) will be discussed later in connection with TLC analysis.

After removing the yellowish aqueous solution, the mass (a quasi-molten phase) of crude lignin was redissolved in a minimum amount of acetone to reprecipitate it in a large excess of distilled water. Yield of the isolated water-insoluble organosolv lignins (FRACTION II) varies between 8.65-31.15% (based on oven-dry extractive-free sawdust), depend-

ing on the cooking conditions as shown in Table 3. More detailed discussion on yield and purity as well as methoxyl content of the isolated lignins will be presented in the following sections.

There are several methods available to purify the precipitated organosolv lignin. However, no purification was carried out because such methods involve dissolution of the crude lignin in certain organic solvents for reprecipitation. This may affect the structure of lignin molecules due to solvent effects. Most of the tests for the present study were conducted on either acetylated or reduced organosolv lignins which can be considered to be purified (freed from sugars) during the reactions and work-up processes.

There is a possibility that the crude lignins from the spent liquor might have been isolated together with hemicellulose fractions. The sugars could have been covalently bonded to lignin molecules or trapped in the tridimensional lignin matrix (26,97,98,141). This possibility will be discussed later in conjunction with microanalysis of the isolated organosolv lignins.

From a preliminary experiment on moisture hysteresis of the isolated organosolv lignin samples, it was learned that they picked up about 12-16% moisture when the samples were exposed to saturated air condition in an Amineco cabinet (31°C/96-98% RH). At GTH room condition (21°C/50% RH), the moisture content of these lignin samples was found to be 4.43-4.81%, which is about half of that of Douglas-fir sawdust (9.44%) under identical conditions. In order to prevent any potential moisture effect, the isolated organosolv lignin samples were kept dry in a phosphoric anhydride desiccator at 50°C. Under such drying conditions, less than 1% moisture content was obtained within 12 hours.

### 5.3. Effect of Cooking Conditions on Yields of Fiber Residue and Lignin Fractions

Among the factors affecting the out-come of organosolv cooking, only cooking time and concentration of acid catalyst were chosen as cooking variables. Other important cooking conditions, such as temperature, cooking liquor composition and wood/liquor ratio, were kept constant because optimum conditions for these variables were worked out in previous studies (36,37,129) under similar or identical conditions as used in the present study. It was found that the cooking temperature had a profound effect on the rate of hydrolysis of Douglas-fir sawdust. For example, the fiber residue yield after 20-min cooking at 160°C was 63.78%, whereas the values obtained at 180° and 200°C after the same period of cooking were markedly reduced to 41.88% and 6.50%, respectively (37). In order to obtain high lignin yields, 200°C was chosen as constant cooking temperature for this study. The effects of temperature and concentration of acid catalyst on the rate of hydrolysis were found to be interchangeable to some extent, i.e. a low acid concentration can be offset by raising the cooking temperature and vice versa (37).

Acetone/water ratio of the organosolv cooking liquor system and wood/liquor ratio were 3:2 by volume and 1:10 by weight, respectively, as mentioned before.

Earlier, a significant effect of particle size of Douglas-fir sawdust on pulp yield and Kappa number was reported (34). To eliminate the extremely fine and coarse particles, only the 10-40 mesh fraction of the sawdust collected was selected as cooking material.

Since moisture content is one of the most important factors

affecting the rate of liquor penetration (37), the extractive-free sawdust samples were kept in the CTH room to maintain a constant moisture content (9.44%) before cooking.

### 5.3.1 Effect of cooking time

As Tables 3 and 4 show, cooking time seems to be the most significant single parameter in regulating the results of the cooks. In general, longer cooking time gives lower fiber yield and higher recovery of precipitable lignin (Fig.9).

In a preliminary cooking experiment (39), under similar cooking conditions as used in the present study, it was found that the fiber yield for long cooks (20 min) was only one-quarter to one-third of that for short cooks (7 min). On the other hand, lignin recovery from long cooks was about 2 to 5 times of that from short cooks. These trends were found to hold for all wood species studied as illustrated in Table 4.

In Table 3, it can be seen that the total lignin content accounted for in cook nos. 15, 18, 19 and 20 were higher than the potential lignin content (31.81%). It is believed that substantial amounts of hemicelluloses removed from wood were isolated together with the lignin fraction (26). Hydrolysis, which aids the dissolution of lignin into the cooking liquor, also occurs at aryl-glycoside bonds of lignin-saccharidic complex during the acid-catalyzed cooking (98,141).

In earlier studies (36,37), it was found that about one-third of the lignin and a large fraction (71%) of hemicelluloses were dissolved in the first 5 minutes during the hydrolysis of Douglas-fir

Table 3. Effect of cooking conditions on yields of fiber residue and lignin fractions from extractive-free Douglas-fir sawdust.

Cook No.	Cooking time (min)	Acid(HCl) concentration(N)	Yield of fiber residue(%) <sup>a</sup>	Yield of lignin-free pulp (%) <sup>a</sup>	Yields of lignin fractions(%) <sup>a</sup>			
					Residual lignin, FRACT'N I	Isolated lignin, FRACT'N II	Water-soluble lignin, FRACT'N III	Total lignin accounted
1	5	-	-	-	-	-	-	-
2	9	-	92.06	-	-	-	-	-
3	12	-	90.16	-	-	-	-	-
4	17	-	86.09	-	-	-	-	-
5	20	-	83.14	-	-	-	-	-
6	5	0.025	69.03	50.99	18.04	8.65	1.20	27.89
7	9	0.025	33.74	27.70	6.04	14.95	1.94	22.93
8	12	0.025	12.94	11.17	1.77	18.45	2.66	22.88
9	17	0.025	11.71	10.13	1.58	25.00	2.27	28.85
10	20	0.025	9.97	8.67	1.30	26.20	2.55	30.05
11	5	0.05	67.02	49.81	17.21	9.12	1.10	27.43
12	9	0.05	29.37	24.18	5.19	17.80	2.04	25.03
13	12	0.05	13.75	11.82	1.93	23.65	2.25	27.83
14	17	0.05	6.36	5.55	0.81	26.95	2.25	30.01
15	20	0.05	3.03	2.59	0.44	29.12	2.69	32.29
16	5	0.1	64.45	48.65	15.88	9.85	1.11	26.84
17	9	0.1	22.54	19.08	3.46	23.40	2.08	28.92
18	12	0.1	7.11	6.23	0.88	28.25	2.76	31.89
19	17	0.1	3.88	3.42	0.46	30.30	2.33	33.09
20	20	0.1	2.47	2.18	0.29	31.15	2.66	34.10

(Cooking temperature=200°C; Wood/liquor ratio=1:10 by weight)

<sup>a</sup>Percentage values based on oven-dry extractive-free sawdust.

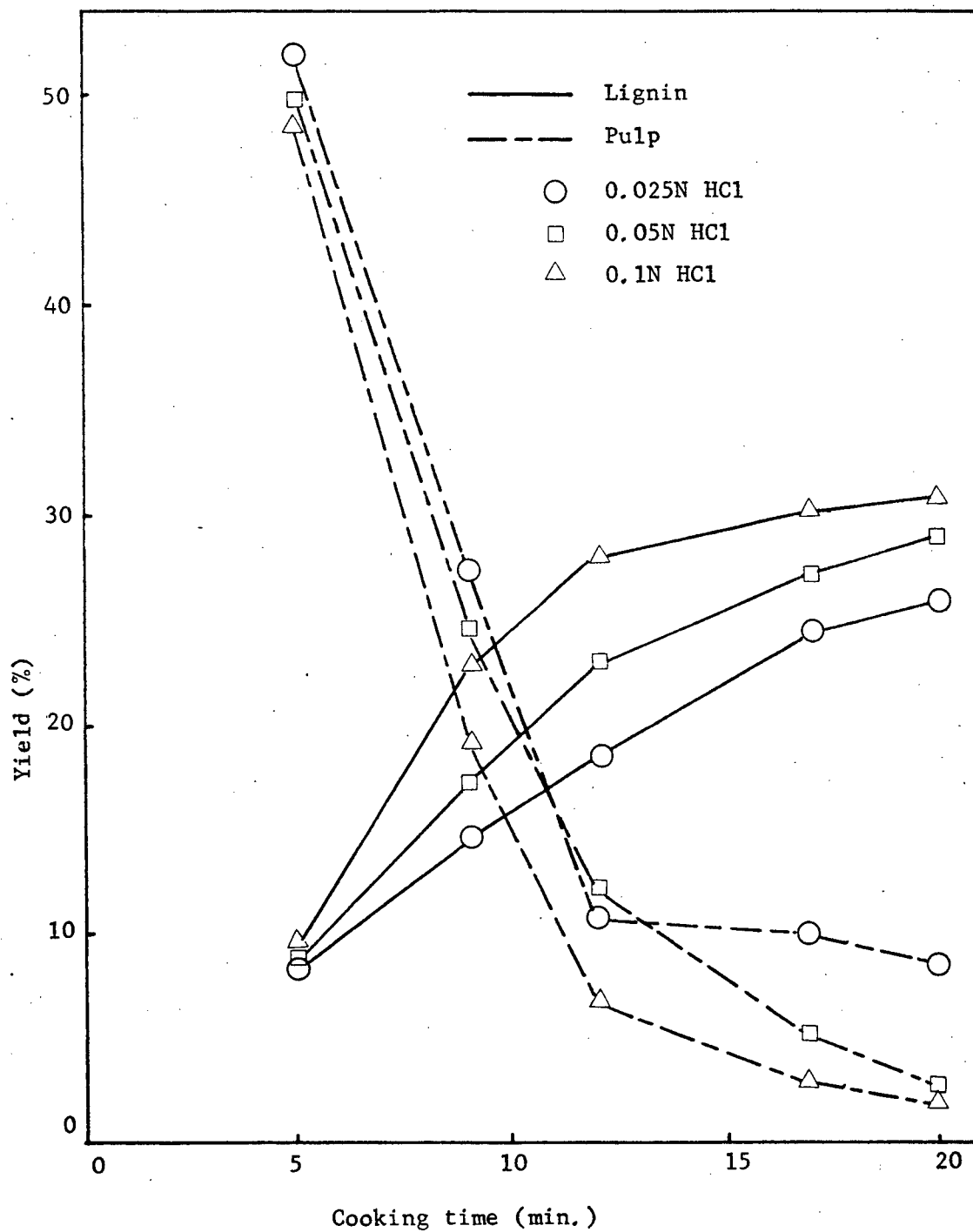


Figure 9. Effect of cooking time on yields of pulp and isolated lignin (FRACTION II) from extractive-free Douglas-fir sawdust.



Table 4. Effect of cooking time on yields of fiber and water-insoluble lignin from various wood species<sup>a</sup> - preliminary cooking experiment(39).

Species	Cooking time (min.)	Fiber yield <sup>b, c</sup> (%)	Lignin yield <sup>b</sup> (%)
Spruce	20	19.78	19.94
	7	57.97	9.74
Douglas-fir	20	14.44	24.78
	7	63.53	5.74
Aspen	20	23.78	16.34
	7	57.88	6.89
Birch	20	12.68	18.36
	7	49.44	8.44
Sugar cane	20	6.45	9.37
	7	38.23	2.61

<sup>a</sup>Wood samples were not pretreated to remove extractives.

<sup>b</sup>Percentage values based on oven-dry wood samples.

<sup>c</sup>Yields were not corrected for residual lignin.

sawdust by acidified organosolv cooking under identical cooking conditions as those used in the present study. In a similar organosolv cooking experiment on Eucalyptus viminalis, Gomide (80) also reported that about 60% of hemicellulose monomeric units and more than one-third of the lignin were removed in the initial pulping stage. In the present study, it was found that about 10-13% of lignin, which is equivalent to about one-third of the total lignin content (31.81%), was released from the wood during the initial 5-min period of the delignification process (Table 3). The temperature at this stage was only 175°C (Fig. 10). The amount of hemicellulose dissolved within this period was about 21-22.5%, which is equivalent to about 83.5-89.5% of the total potential hemicellulose content of 25.15% (Table 2).

Even though the bulk of lignin and hemicelluloses were removed during 5-9 minutes of cooking, samples of cook nos. 6, 7, 11, 12, 16 and 17 seem to be incompletely delignified. Residual lignin content of these cooks ranged between 3.46-18.04%, based on the oven-dry extractive-free sawdust (Table 3). The high residual lignin content in short cooks might have been caused by experimental errors in Kappa number determination because the residual fibers could not be blended before the addition of 0.1N potassium permanganate solution. As a result, the standard reaction time (10 min) might have been too short to complete the reaction, resulting in lower consumption of 0.1N potassium permanganate solution, and thus giving erroneous Kappa numbers. Another possible explanation for the high content of the residual lignin may be the fact that the decomposed sugars condensed with lignin and precipitated on the fiber residue during the organosolv cooking. However, no visual evidence of this was found.

It is also known that cellulose degradation is time dependent (3). After 20-min cooking, cook no. 15 (20 min; 0.05N HCl) and cook

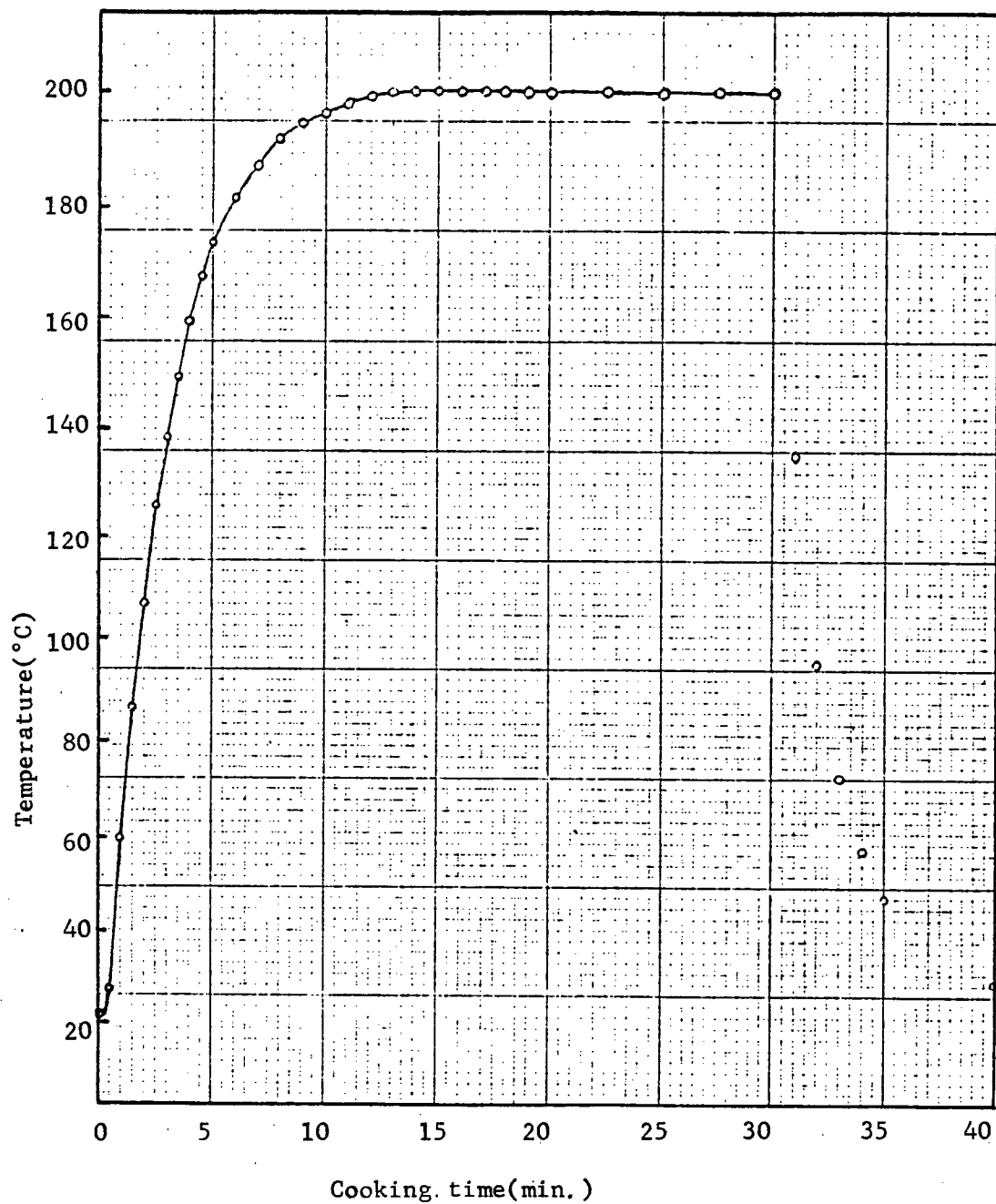


Figure 10. Cooking-bomb temperature vs. cooking time.

no.20 (20 min;0.1N HCl) gave almost total dissolution of the wood constituents leaving less than 3% of the starting material as fiber residue (Table 3 and Fig.9). When the cooking temperature reached 200°C, after about 11-12 minutes (Fig.10), the maximum pressure registered about 320 psig and was stabilized. Maximum pressure in the organosolv cooking system was obtained faster (6-7 min) than the maximum temperature.

### 5.3.2 Effect of acid catalyst concentration

It was found that the increase of acid catalyst concentration generally increased the rate of delignification in organosolv pulping (36,153). This is attributed to the faster acidolytic splitting of the lignin-carbohydrate complex into fragments small enough to be soluble in the aqueous organic cooking system (97).

Cooking results presented in Table 3 show that fiber residue yield decreased and the isolated lignin (FRACTION II) yield increased as the acid concentration increased from 0.025 to 0.1 N HCl. This general observation can be observed in Fig. 11. The effect of acid concentration on yields of fiber residue and the organosolv lignin, however, is not as significant as that of cooking time (Fig. 9). The increased catalyst level seems to cause rapid dissolution of polysaccharides as well as rapid delignification because both phenomena must be regarded essentially as the hydrolytic solvolysis process of wood (153).

At a high acid concentration (0.1N), extensive dissolution of the wood constituents, beyond the amount represented by hemicelluloses and lignin, occurred. As mentioned before, cook nos.18 (0.1 N HCl;12 min), 19 (0.1 N HCl;17 min) and 20 (0.1 N HCl;20 min) produced more lignin than the potential lignin content (31.81%) possibly due

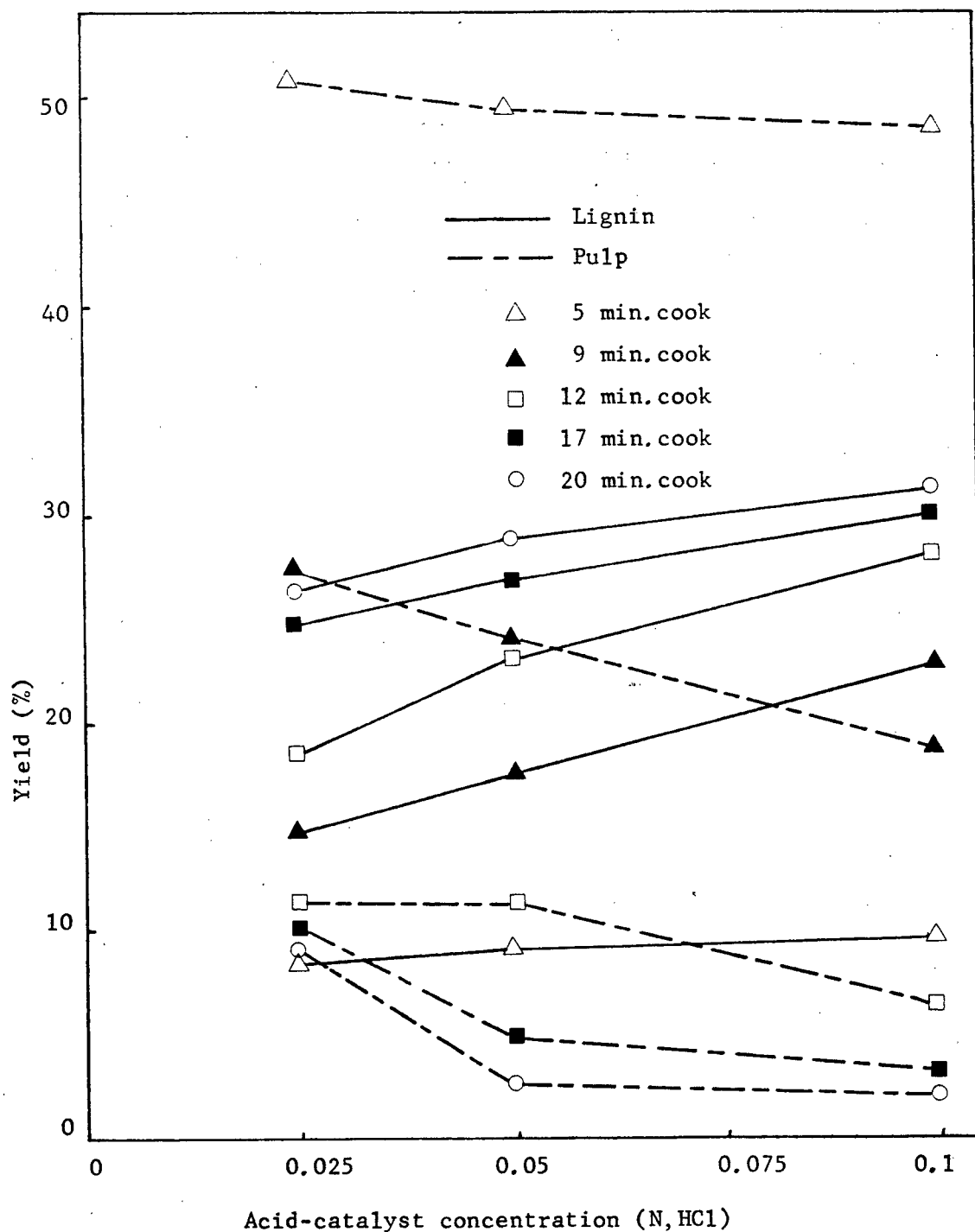


Figure 11. Effect of acid concentration on yields of pulp and isolated lignin (FRACTION II) from extractive-free Douglas-fir sawdust.

to contamination by hemicelluloses (26). Cook no. 15, having been made at an intermediate acid concentration of 0.05 N, gave similar results due to the long cooking time (20 min), leading to nearly total dissolution of wood and thus resulting in a mere 2.59% fiber residue yield and nearly quantitative recovery of the precipitable lignin.

The pH value of pure acetone-water cooking liquor was about 6 and those of 0.025, 0.05 and 0.1 N hydrochloric acid solutions were found to be 2.5, 1.5 and 1.2, respectively. Table 5 shows the change of pH values after 20-min cooking. This change seems to be due to an accumulation of organic acids, such as acetic acid and formic acid, liberated from the wood during cooking (36). Though such organic acids can affect the rate of delignification (94), their effect was found to be insignificant. In the presence of a strong mineral acid such as hydrochloric acid, their effect was completely unnoticed and unimportant in spite of the fact that substantial decreases in the final pH of the cooking liquor was noticed (Table 5).

In the absence of acid catalyst, the rate of hydrolytic dissolution was obviously very slow, even though the pH of the cooking liquor had been lowered to 3.2 at the end of a 20-min cook from the near neutral starting pH as shown in Table 5. The amount of lignin extracted from this series (cook nos. 1-5) was too small to isolate, therefore no further analysis was attempted. By a simple extraction with organic solvents, it was found that only about 1% of the original lignin can be extracted (137). This series (cook nos. 1-5; without catalyst) proves that the delignification process is not just a solvolysis process, but requires sufficient strength of catalyst for the hydrolysis reaction, if substantial amount of lignin are to be removed from the wood in the course of high temperature organosolv cooking.

Table 5. pH values of acid-catalyzed cooking liquor<sup>a</sup> before and after 20-min cooking.<sup>b</sup>

HCl concentration(N)	before cooking	after cooking
0	6.0	3.2
0.025	2.5	2.2
0.050	1.5	1.2
0.100	1.2	1.1

<sup>a</sup>Cooking liquor:acetone/water=60/40, by volume.

<sup>b</sup>Cooking with extractive-free Douglas-fir sawdust(wood/cooking liquor =1/10)

#### 5.4 Water-soluble Lignin Fraction

It was not the intention of the present study to discuss in detail the water-soluble lignin (FRACTION III), but it must be mentioned that some important observations were made with regard to this fraction.

As mentioned before, repeated attempts to isolate the water-soluble lignin fraction in the solid state had failed. Even quantitative separation of this fraction from aqueous filtrates of the spent liquor by liquid-liquid extraction was found to be somewhat a problem since the solubility of this fraction in water was not very much lower than that of chloroform or that of other water-immiscible organic solvents. The semi-quantitative determination of the water-soluble lignin by UV spectrophotometry also provided somewhat questionable results (Table 3). In any cook, less than 3% of the extractive-free sawdust was detected as water-soluble lignin.

However, it was found that as cooking time increased, the yield of the water-soluble lignin fraction seemed to increase. For example, increasing the cooking time from 5 to 20 minutes almost doubled the yield of water-soluble lignin fractions in all cases. Peculiarly, maximum yield of these fractions occurred for the 12-min cooks with only a slight fall-off for 20-min cooks, indicating a fair degree of thermal stability of water-soluble lignins. Effect of increasing concentration of acid catalyst on yield of water-soluble lignin seems to be insignificant.

For the lack of an adequate isolation method, an accurate overall lignin mass-balance could not be obtained. The quantitative determination of this fraction, however, will be discussed in connec-



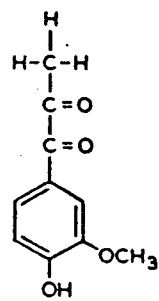
tion with TLC analysis in the following section.

#### 5.5 Thin-Layer Chromatographic Analysis of Water-soluble Lignin Fraction from Spent Cooking Liquor

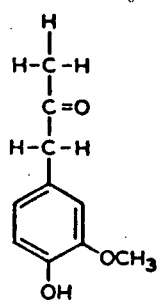
Regarding the lignin mass-balance, it is evident that considerable amounts of lignin and lignin-degradation products were present as water-soluble substances in the spent cooking liquor. Reactions that convert protolignin into water-soluble derivatives have been the subject of numerous investigations. Hibbert and his co-workers (27,46,119) were the first group to investigate these water-soluble materials from the ethanolysis of lignin. A number of water-soluble compounds which were found to be monomeric phenylpropane  $C_9$ -units were isolated, identified and assumed to be lignin-degradation products(26, 131).

The organosolv cooking of extractive-free sawdust in acid medium is essentially an acid hydrolysis. It was reported that aqueous hydrolysis liquors of softwood contain low molecular weight aromatic materials such as coniferyl alcohol (II), vanillic acid (XIII), vanillin (XVI), acetyl vanilloyl (XXI) and guaiacyl acetone (XXII) as well as some Hibbert's ketones (26,97,131). Those compounds which are likely present in the aqueous fraction of the spent cooking liquor of the present study are listed in Table 6 along with the known  $R_f$  values and characteristic colors on silica gel TLC (13,70,100).

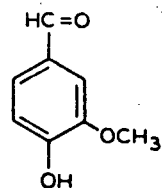
Table 7 presents TLC results of the aqueous fraction from cook no.15 (20 min;0.05 N HCl) after removing the sugars. Among the compounds identified were Hibbert's ketones such as acetyl vanilloyl (XXI), guaiacyl acetone (XXII),  $\alpha$ -hydroxypropiovanillone (XXVII) and



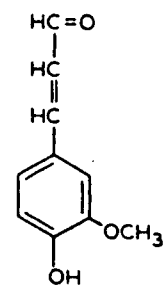
(XXI)



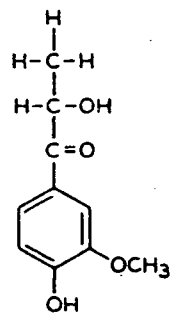
(XXII)



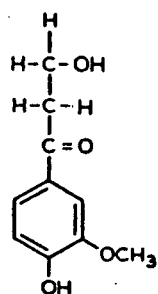
(XVI)



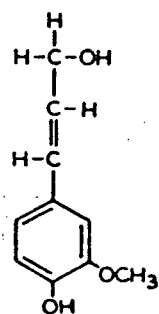
(XXVI)



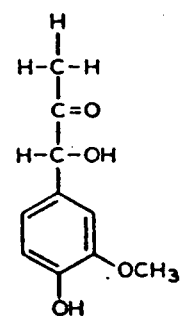
(XXVII)



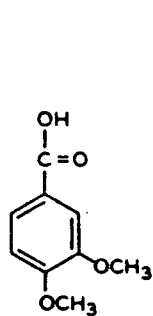
(XXVIII)



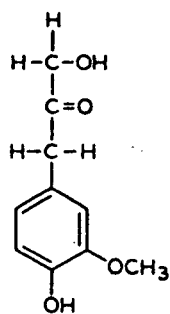
(II)



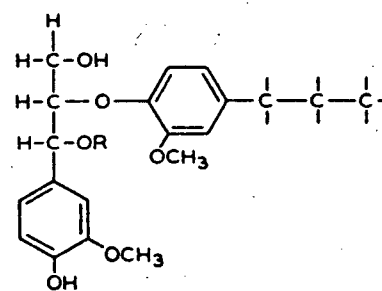
(XXIX)



(XIII)



(XXIV)



(XXIII)

Table 6.  $R_f$  values and characteristic colors of selective lignin degradation products and extractives(31,70,100).

Compound	$R_f \times 100$				Color on silica gel	
	B-C-M <sup>a</sup>	B-E <sup>b</sup>	B-A <sup>c</sup>	M-C <sup>d</sup>	Folin-Denis Reagent	Diazotized sulfanilic acid
Acetyl vanilloyl(XXI)	53	47	52	83	dark brown	yellow
Guaiacyl acetone(XXII)	49	44	60	93	mustard	brown
Vanillin(XVI)	48	40	59	93	orange-red	red
Coniferyl aldehyde(XXVI)	42	47	55	93	wine-red	red-brown
$\alpha$ -Hydroxypropiovanillone(XXVII)	36	30	37	77	yellow	brown
$\beta$ -Hydroxypropiovanillone(XXVIII)	31	20	32	77	brown	brown
Coniferyl alcohol(II)	28	37	40	76	blue	red-brown
$\alpha$ -Hydroxyguaiacyl acetone(XXIX)	-	-	-	-	-	-
Vanillic acid(XIII)	25	23	17	42	pale yellow	yellow-orange
$\omega$ -Hydroxyguaiacyl acetone(XXIV)	-	32	43	70	-	pink
Guaiacylglycerol- $\beta$ -guaiacyl ether (XXIII)	17	-	-	-	yellow	-

<sup>a</sup> Benzene-chloroform-methanol(70:28:2)

<sup>b</sup> Benzene-ethanol(150:22)

<sup>c</sup> Benzene-acetone(3:2)

<sup>d</sup> Methanol-chloroform(3:7)

Table 7. Thin-layer chromatography of water-soluble fraction from organosolv spent liquor(after removal of carbohydrates).

Cpd. No.	Tentative identification	$R_f \times 100$			
		B-C-M <sup>a</sup>	B-E <sup>b</sup>	B-A <sup>c</sup>	M-C <sup>d</sup>
1.	Acetyl vanilloyl(XXI)	53	49	52	89 <sup>e</sup>
2.	Guaiacyl acetone(XXII)	50	43	62	94 <sup>e</sup>
3.	Vanillin(XVI)	47	41	58	92 <sup>e</sup>
4.	Coniferyl aldehyde(XXVI)	44	45	54	90 <sup>e</sup>
5.	$\alpha$ -Hydroxypropiovanillone(XXVII)	36	29	32	79
6.	$\beta$ -Hydroxypropiovanillone(XXVIII)	31	19	27	74
7.	Coniferyl alcohol(II)	?	37	39	?
8.	Vanillic acid(XIII)	25	?	?	47
9.	$\omega$ -Hydroxyguaiacyl acetone(XXIV)	?	?	?	66
10.	Guaiacylglycerol- $\beta$ -guaicyl ether(XXIII)	17	?	7	32
11.	Undeveloped starting material & Furfural(XXX)	2	3	2	11

<sup>a</sup> Benzene-chloroform-methanol(70:28:2)

<sup>b</sup> Benzene-ethanol(150:22)

<sup>c</sup> Benzene-acetone(3:2)

<sup>d</sup> Methanol-chloroform(3:7)

<sup>e</sup> Partially overlapped.

$\beta$ -hydroxypropiovanillone (XXVIII). The presence of these ketones is a good indication of lignin-degradation by cleavage of ether bonds during the acid-catalyzed organosolv cooking.

From the data presented in Table 7, it can be seen that beside the Hibbert's ketones, lignin molecules further proportioned into degradation products such as vanillin (XVI) and coniferyl aldehyde (XXVI), even though the amounts of these fractions seems to be small. Kratzl and Paszner (100) reported that simple aqueous hydrolysis of wood at 100°C for 2-4 hours also yields these compounds in somewhat larger proportions.

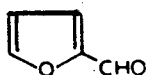
TLC results of cook nos. 11<sup>a</sup> (5 min; 0.05 N HCl) and 13 (12 min; 0.05 N HCl) were found to be similar to those of cook no. 15 (20 min; 0.05 N HCl), indicating that the degradation of lignin molecules takes place to about the same extent during the initial delignification period of organosolv cooking as mentioned before. This observation is in good agreement with the results obtained from NMR spectra which will be discussed later.

The presence of coniferyl alcohol (II), vanillic acid (XIII) or  $\omega$ -hydroxyguaiacyl actone (XXIV) could not be confirmed due to the absence of the corresponding spots in the developing systems used (Table 7). For the same reason, guaiacylglycerol- $\beta$ -guaiacyl ether (XXIII), which is primarily responsible for formation of Hibbert's ketones, could not be identified. Notable missing compounds are benzyl alcohols, presumably because of their instability under acidic cooking conditions. Their self-condensation in the acid medium is well known (141).

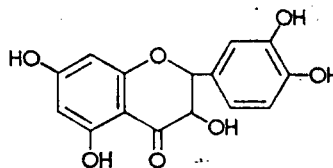
Inasmuch as all of the compounds mentioned above are phenolic in nature, it appeared that hydrolysis of the non-carbohydrate portion of wood reduced part of the lignin molecule to low molecular degradation

products (131). The formation of phenolic hydroxyl groups as result of splitting of  $\beta$ -aryl ether bonds of lignin molecules is another important factor for the increased solubility of lignin-degradation products in aqueous solution.

Not surprisingly, traces of furfural (XXX), which is a sugar-degradation product (36), were also detected. There were some spots which could not be easily identified. These spots might have originated from some extractives, such as dihydroquercetin (XXXI), because some extractive deposits in lumens were reported to resist alcohol-benzene extraction (33,91).



(XXX)



(XXXI)

Since water-soluble lignin or lignin-like compounds were not the prime target of the present study and insufficient amounts of the water-soluble fraction were obtained, no further analysis was carried out as mentioned before.

## 5.6 Microanalysis of Isolated Organosolv Lignins

Lignin contains only carbon, hydrogen and oxygen, and the elementary compositions reported in the literature show considerable variation because of the variety of sources and methods in lignin preparation.

The carbon content of softwood lignins are in a range of 60.2-67.5% and the corresponding hydrogen content ranges 4.5-6.4% (32).

As shown in Table 8, carbon content of the acetylated Douglas-fir lignin samples ranged from 62.99 to 67.66%, while hydrogen contents were 4.80 to 6.14%. Average elementary composition of Douglas-fir MWL from normal wood are reported to be 63.37% carbon, 6.07% hydrogen and 30.56% oxygen (148).

In Table 8, it can be seen that methoxyl group content of the acetylated lignin generally decreased as both cooking time and acid concentration increased. The reason for this observation may be explained by the association of the isolated lignin (FRACTION II) with hemicellulose contaminants (26). As the cooking time or acid concentration increased, the isolated lignin fraction seemed to contain increasingly larger amounts of carbohydrate fragments due to secondary condensation between solubilized lignin and carbohydrate fragments. As a result the methoxyl content decreases significantly.

However, there are strong points against the secondary condensation between lignin and carbohydrates. As an evidence for this argument, NMR spectra of the same acetylated lignin samples show no signals for hemicellulose contaminants, which will be discussed later. Earlier an attempt to condense lignin and lignin model substances with furfural gave negative results (169). From this it may be concluded that lignin which has been isolated from wood by hydrolysis has undergone chiefly an autocondensation in which the functional groups of the side-chain, the phenolic hydroxyl groups, and the reactive hydrogen atoms of the aromatic rings are involved (169).

There is an alternative explanation for the variation of methoxyl content with cooking period. Protolignin is non-homogeneous

Table 8. Elementary composition of acetylated Douglas-fir lignin samples.

(%)

Sample No. <sup>b</sup>	Carbon	Hydrogen	Oxygen	OCH <sub>3</sub> <sup>a</sup>
PC-11	65.62	5.01	29.37	12.90(14.84)
PC-12	64.25	5.29	30.46	12.42(14.37)
PC-13	64.50	5.22	30.28	12.11(14.18)
PC-14	65.14	4.91	29.95	11.36(13.24)
PC-15	62.64	5.48	31.88	11.18(13.20)
PC-9	65.08	5.11	29.81	12.33(13.54)
PC-19	66.61	5.07	28.32	11.07(12.80)

<sup>a</sup>Values in parentheses are for parent isolated lignin.

<sup>b</sup>Sample number code 'PC' was used to distinguished the treated (acetylated or reduced) samples from their parent lignin samples (FRACTION II).



in terms of methoxyl content (135), and the lignin fractions having higher methoxyl contents are most readily liberated from wood during the organosolv cooking. Thus, short cooks, such as cook nos. 11 (5 min) and 12 (9 min) produced lignins with higher methoxyl contents (14.17-14.64%), while long cooks, such as cook nos. 9 (17 min), 14 (17 min), 19 (17 min) and 20 (20 min) gave lignins with much lower methoxyl contents (11.20-13.54%).

## 5.7 Spectroscopic Analyses of Isolated Organosolv Lignins

### 5.7.1 Nuclear magnetic resonance spectra

The liberation of lignin from wood by acid-catalyzed hydrolysis at high temperature yields lignin with changed chemical structure, even when mild reaction conditions are used (107,137). NMR analysis is one of the best techniques to examine the chemical changes of the lignin molecules caused by the various cooking conditions. Though NMR spectral analysis was done on the acetate derivatives of the isolated (parent) organosolv lignin samples, the discussion of the functional group contents refers to the parent organosolv lignin samples.

A typical NMR spectrum of the acetylated Douglas-fir lignin is shown in Fig. 12. To determine quantitative estimations of the functional groups, several selected ranges of  $\delta$ -value were constructed according to the method used by earlier investigators (57,98,105,107). The NMR spectra of protons in organic compounds can usually be integrated electronically with a high degree of precision. However, the complexity of lignin spectra made it necessary to use the method men-

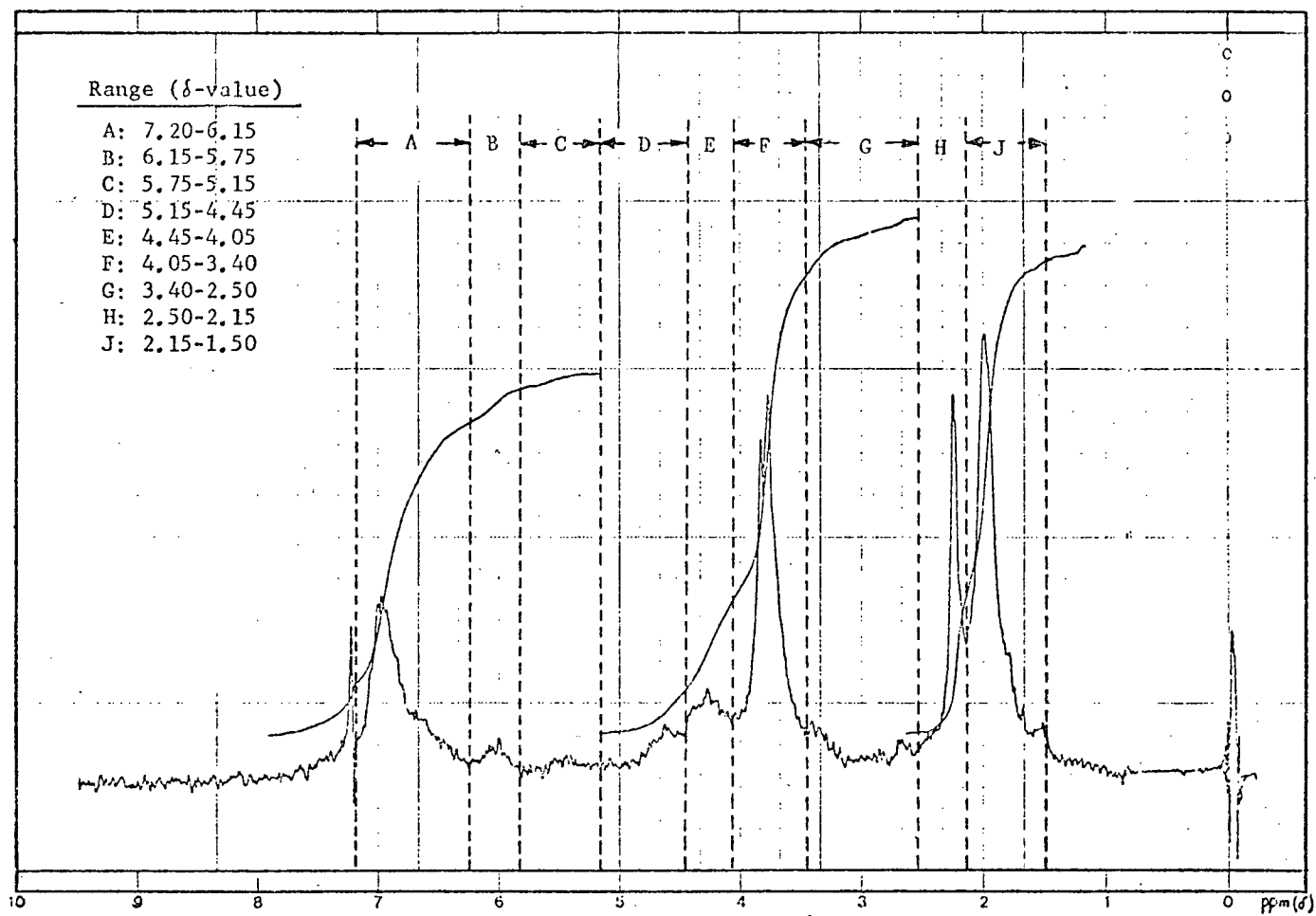


Figure 12. Typical NMR spectrum of acetylated Douglas-fir lignin.

tioned above to determine the percentages of total signal strength found within the selected ranges (107). Table 9 gives the assignments of signals for the selected ranges of the NMR spectrum of the acetylated lignin sample, shown in Fig. 12.

Range A ( $\delta$  7.20-6.15) gives signals for aromatic and  $\alpha$ -vinyl protons. However, since the number of unsaturated structures of the vinyl type in lignin has been shown to be very small, interference from  $\alpha$ -vinyl protons is not appreciable. Fig. 12 clearly shows that the guaiacyl unit around  $\delta$  6.95 predominates over that of syringyl unit around  $\delta$  6.60.

Ranges B ( $\delta$  6.15-5.75), C ( $\delta$  5.75-5.15), D ( $\delta$  5.15-4.45), E ( $\delta$  4.45-4.05) and G ( $\delta$  3.40-2.50) represent signals for  $\beta$ -vinyl protons (and some  $\alpha$ -protons),  $\alpha$ -protons,  $\beta$ -protons,  $\gamma$ -protons and  $\beta$ -protons, respectively.

Range F ( $\delta$  4.05-3.40) shows signals from aromatic methoxyl protons. Ranges H ( $\delta$  2.50-2.15) and J ( $\delta$  2.15-1.50) represent signals for aromatic acetoxyl protons and aliphatic protons, respectively.

Table 10 shows the percentage estimations of the various proton types occurring within the assigned ranges of the integrated spectra (Figs. 13-19). From these values and the results from the microanalysis (Table 8), contents of various functional groups of the parent lignin samples were estimated and the results are presented in Table 11. Due to the lack of microanalysis data on parent lignins, accurate empirical formulae could not be calculated. However, strictly from the NMR spectra and elemental analysis of acetylated lignins, approximate empirical formulae could be constructed (Table 11).

Table 9. Assignments of signals in NMR spectrum of acetylated Douglas-fir lignin sample.

Range		Chemical shift, $\delta$ -value (ppm)	Assignment (types of proton)
Symbol	$\delta$ -value (ppm)		
A	7.20-6.15	6.95	aromatic (also some $\alpha$ -vinylic)
B	6.15-5.75	6.05	$\beta$ -vinylic $\alpha$ -proton of side chain ( $\beta$ -O-4 linkage)
C	5.75-5.15	5.40-5.80	$\alpha$ -proton of side chain ( $\beta$ -5 linkage or benzyl aryl ether)
D	5.15-4.45	4.55	$\beta$ -proton of side chain ( $\beta$ -O-4 linkage)
E	4.45-4.05	4.25	$\gamma$ -proton of side chain
F	4.05-3.40	3.75 (3.81)	methoxyl
G	3.40-2.50	2.60-3.40	$\beta$ -proton of side chain ( $\beta$ - $\beta$ linkage)
H	2.50-2.15	2.25	aromatic acetoxy1
J	2.15-1.50	2.00	aliphatic acetoxy1



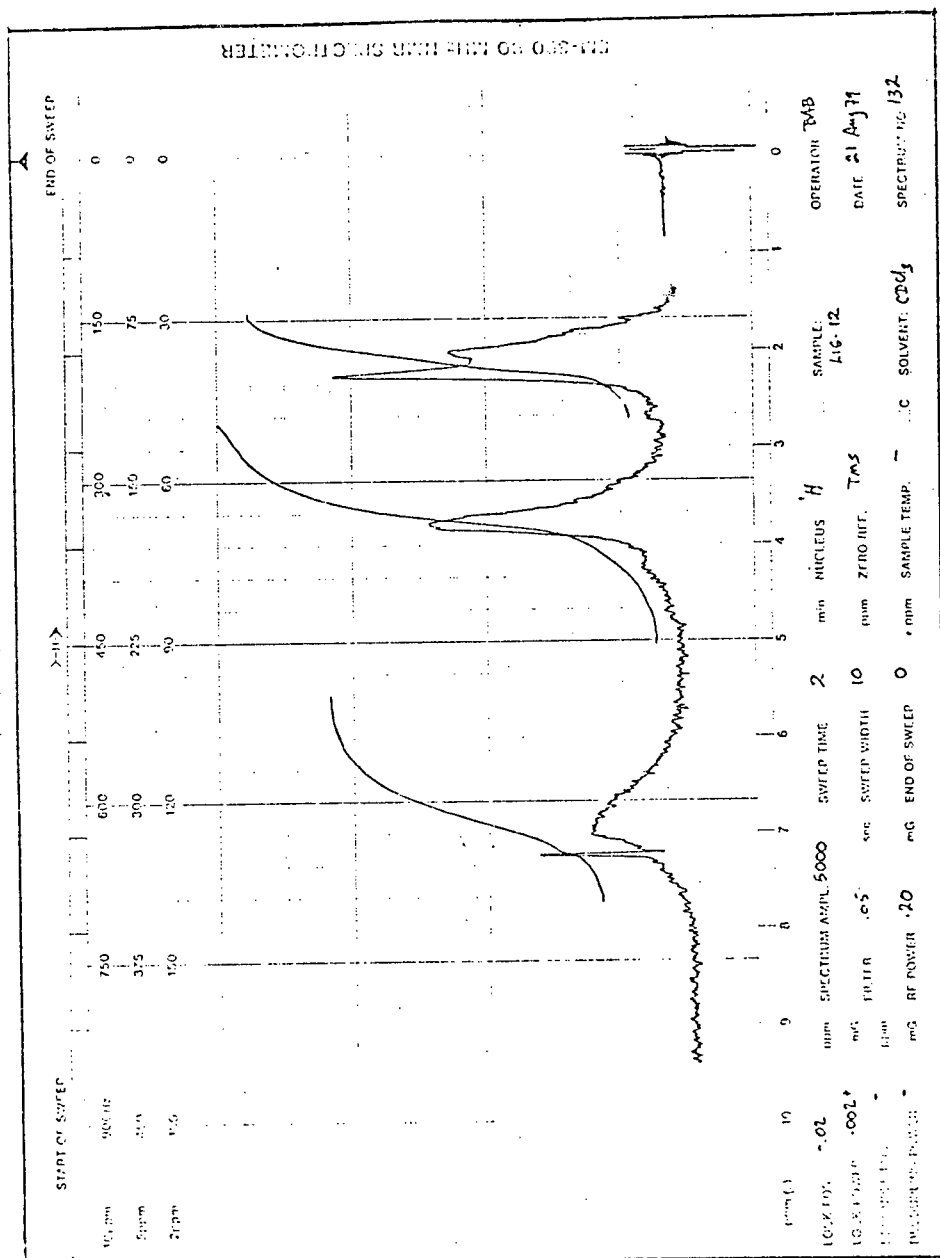


Figure 14. NMR spectrum of sample PC-12.

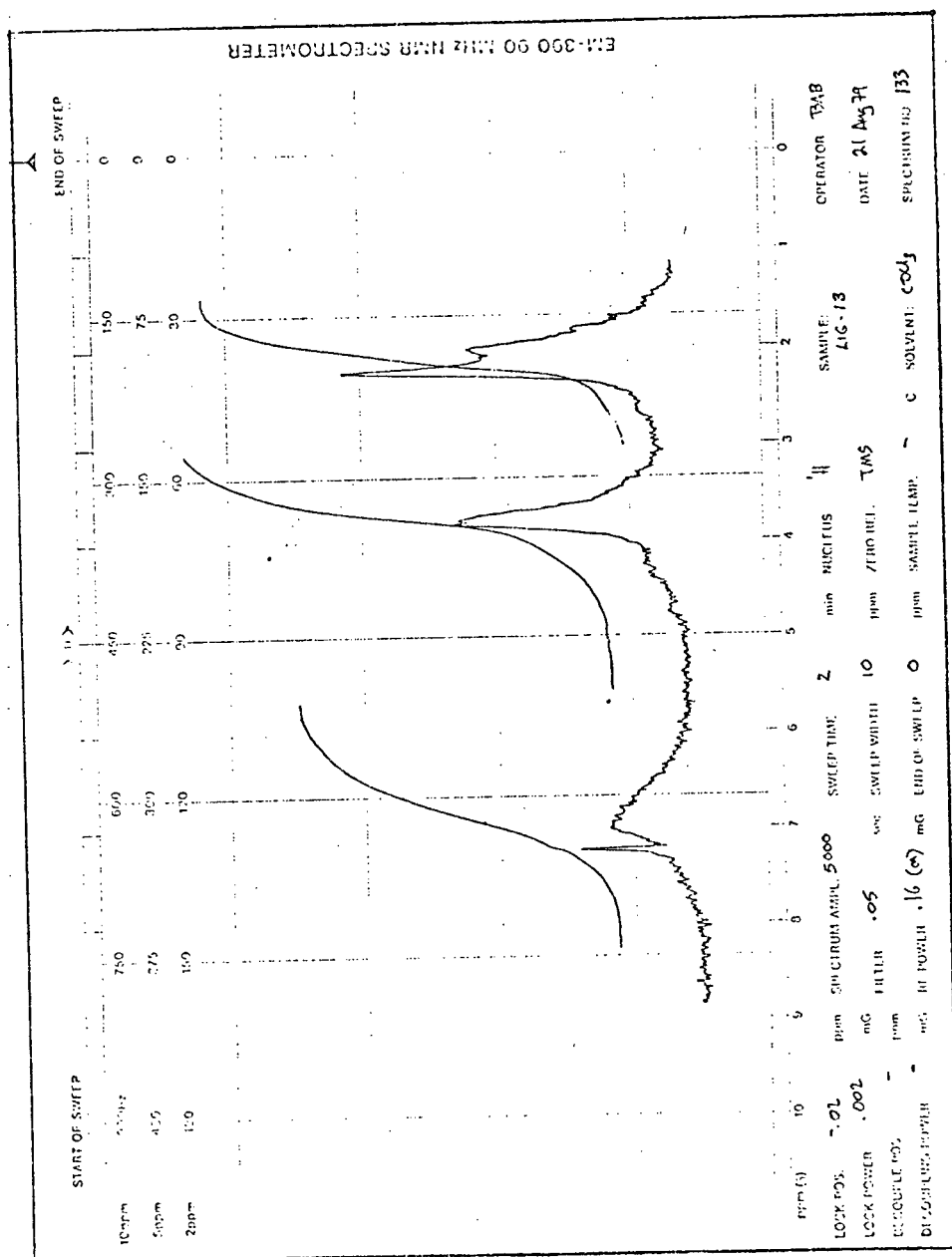


Figure 15. NMR spectrum of sample PC-13.

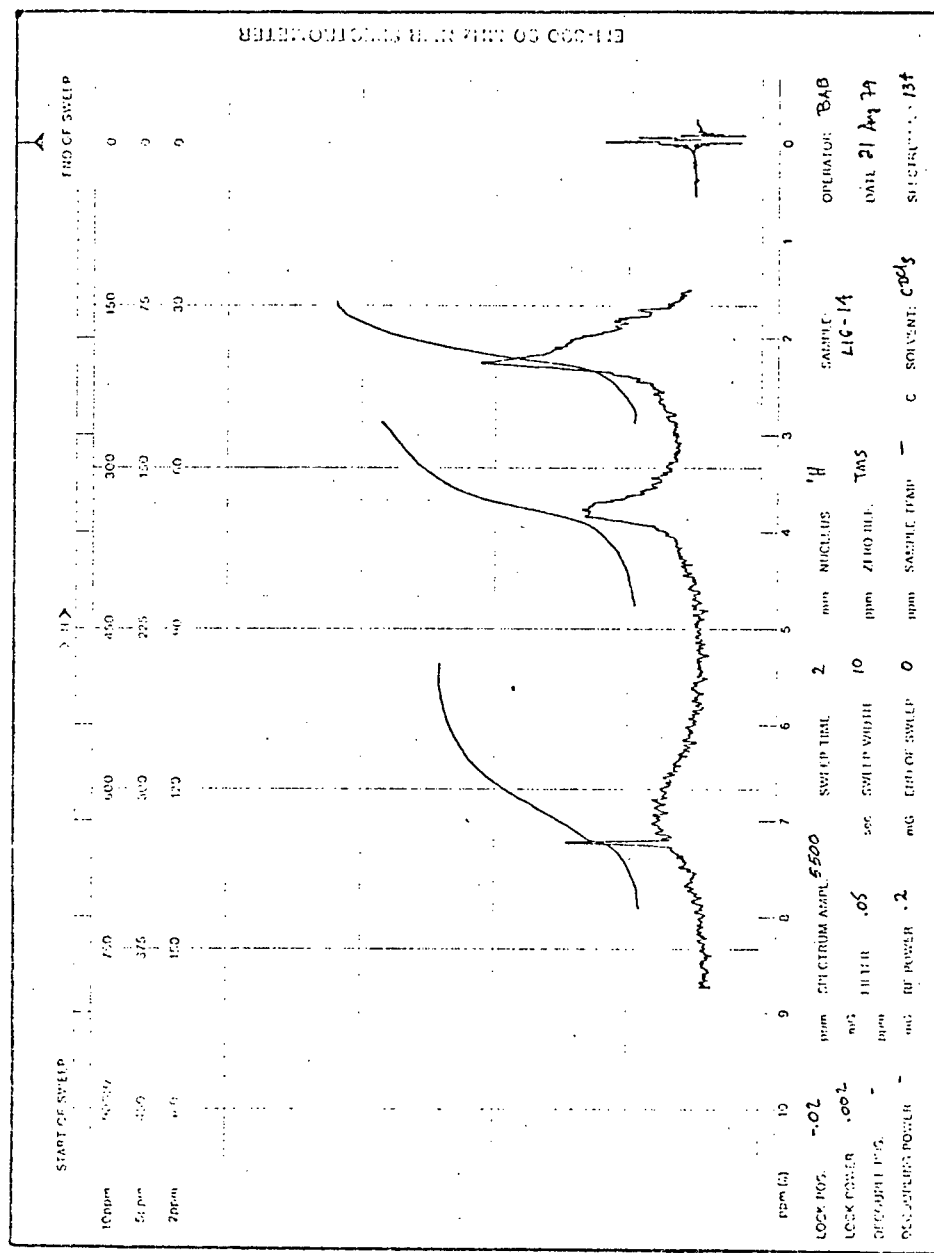


Figure 16. NMR spectrum of sample PC-14.



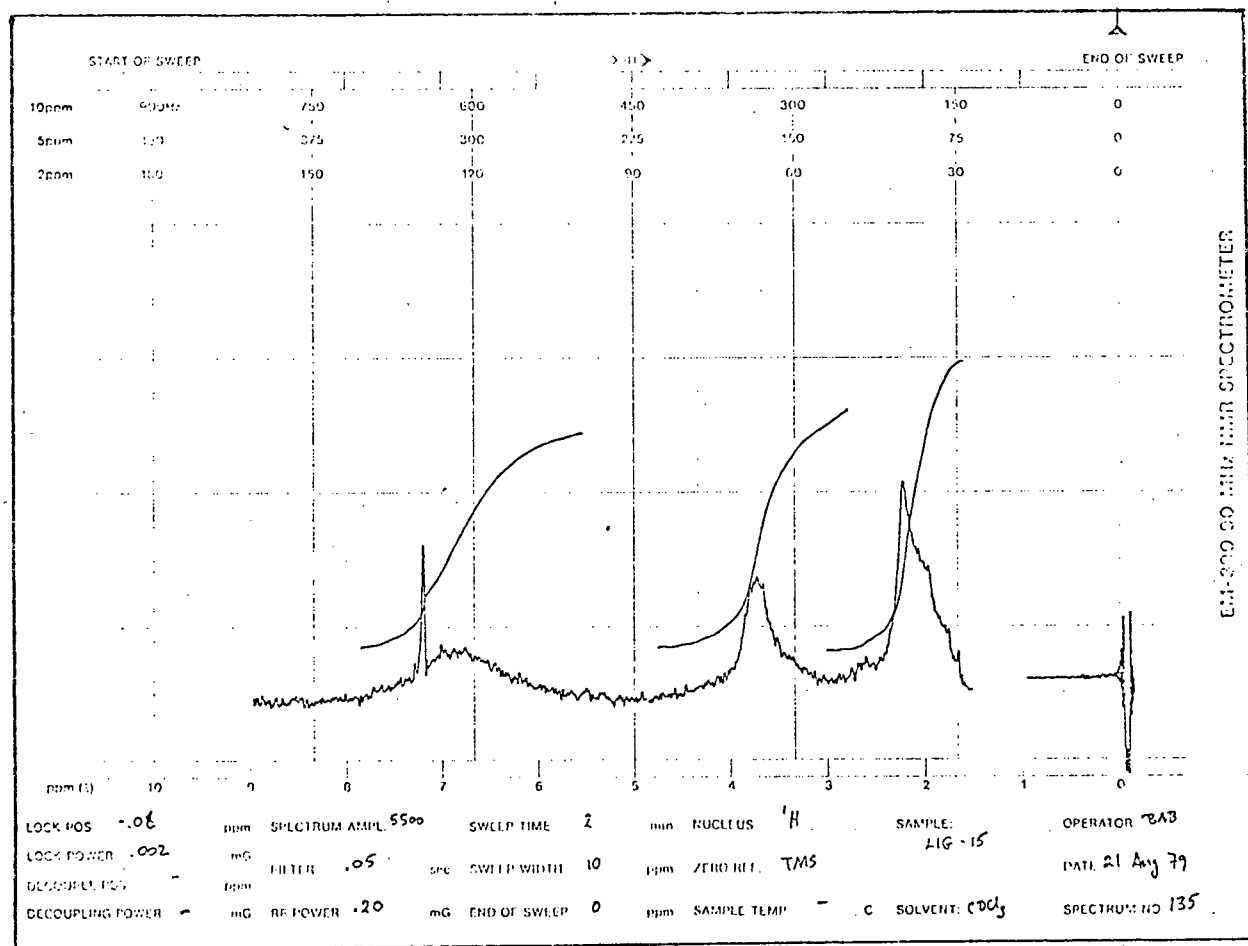


Figure 17. NMR spectrum of sample PC-15.

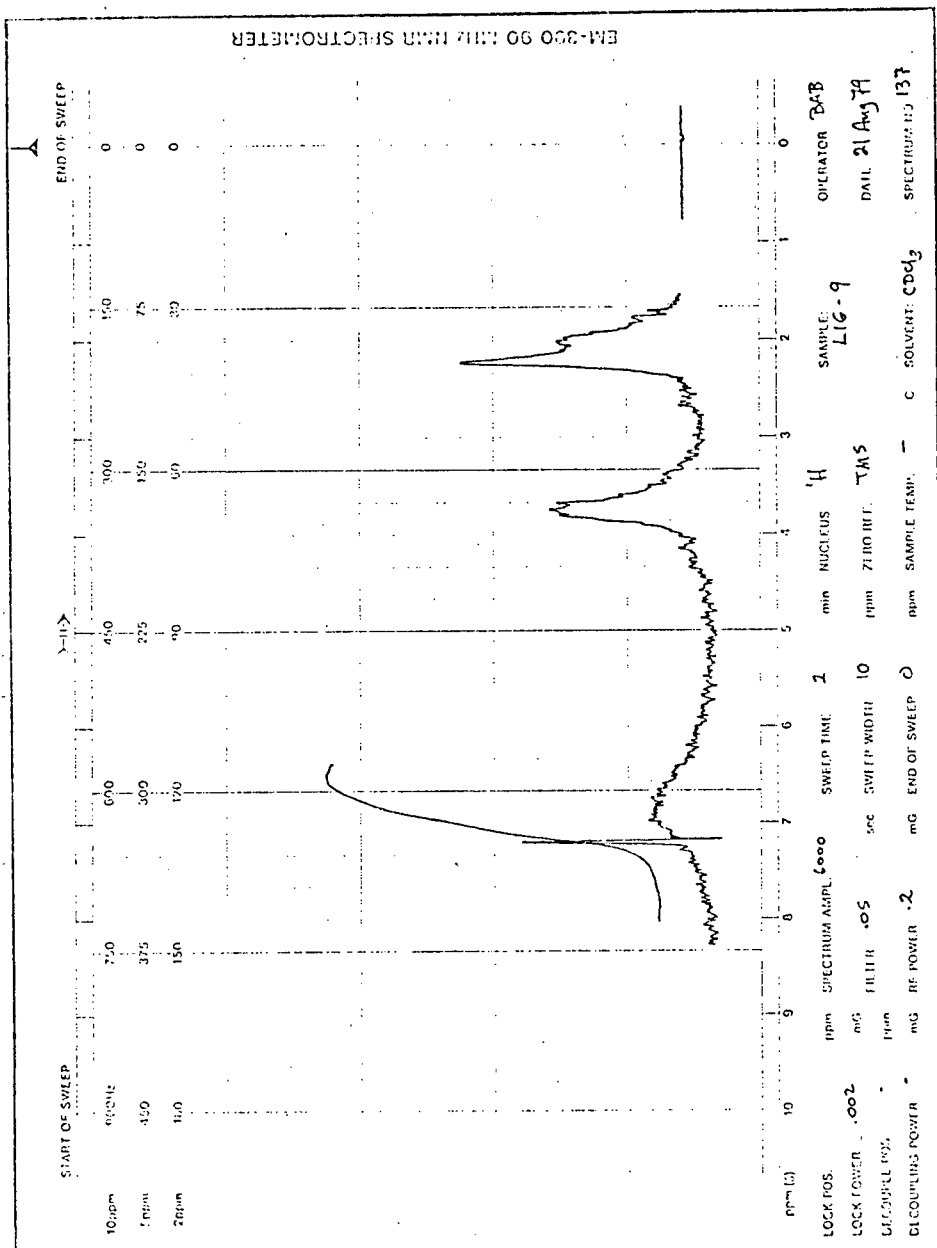


Figure 18. NMR spectrum of sample PC-9.

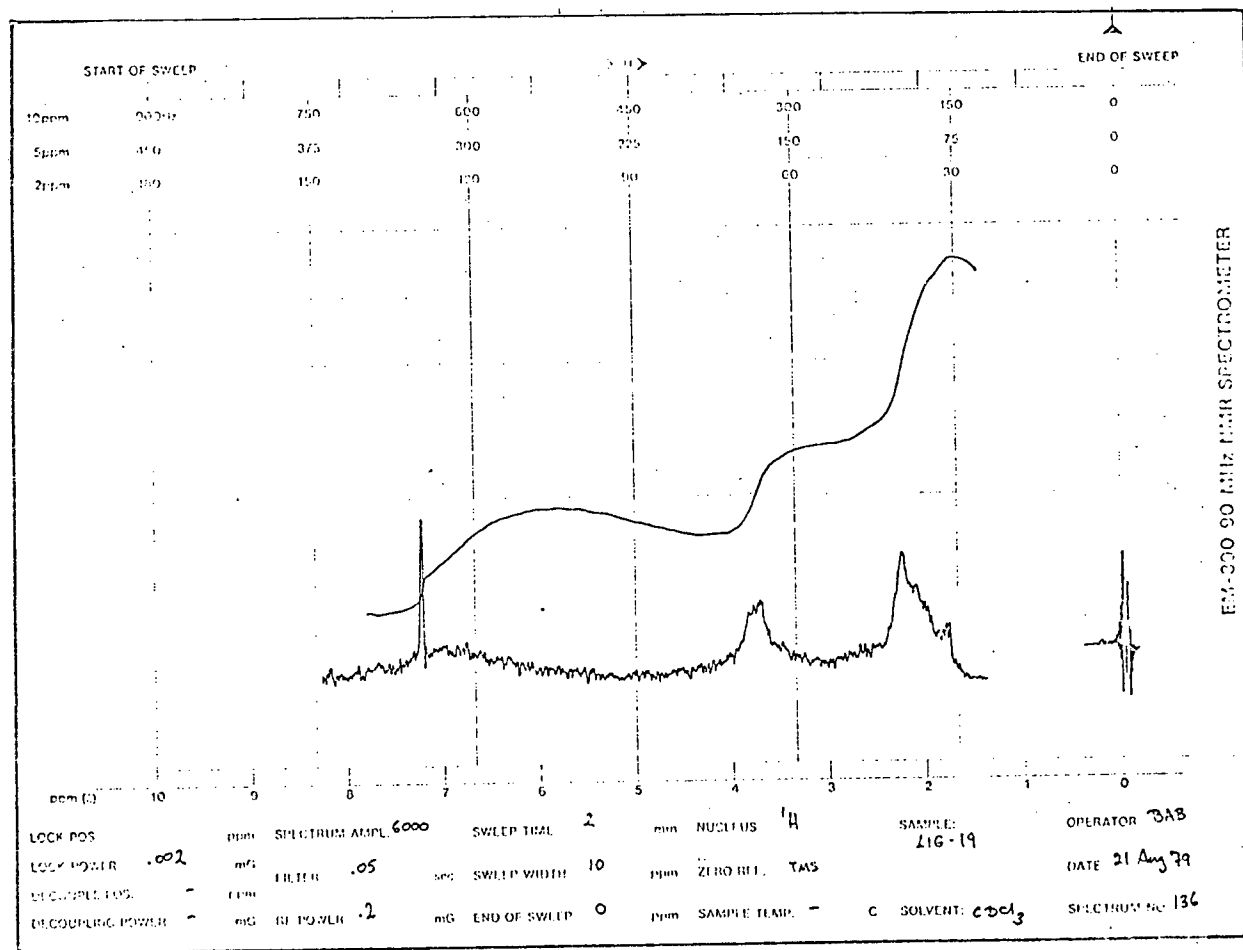


Figure 19. NMR spectrum of sample PC-19.

Table 10. Relative intensity of various proton types in NMR spectra of acetylated lignin samples. (%)

Symbol of range	$\delta$ -value (ppm)	PC-11	PC-12	PC-13	PC-14	PC-15
A	7.20-6.15	20.32	19.87	20.64	20.19	21.57
B	6.15-5.75	2.58	1.30	2.62	2.88	3.85
C	5.75-5.15	0.32	0.65	0.29	0.96	0.49
D	5.15-4.45	2.58	2.93	4.07	5.77	2.94
E	4.45-4.05	8.39	9.77	7.85	9.13	5.88
F	4.05-3.40	24.84	24.10	25.00	23.03	24.02
G	3.40-2.50	5.48	8.14	8.43	9.62	9.31
H	2.50-2.15	10.00	11.07	13.03	(29.33) <sup>a</sup>	(31.85) <sup>a</sup>
J	2.15-1.50	25.48	22.15	18.08		

<sup>a</sup> Combined Ranges H and J together due to overlap.

Table 11. Empirical formulae and functional group contents of isolated lignin samples.

Sample No.	Empirical formula	Apparent Weight of C <sub>9</sub> -unit	Aromatic H		Aromatic OH		Aliphatic OH		OCH <sub>3</sub>	
			per C <sub>9</sub>	%	per C <sub>9</sub>	%	per C <sub>9</sub>	%	per C <sub>9</sub>	%
PC-11	C <sub>9</sub> H <sub>4.63</sub> O <sub>0.56</sub> (OCH <sub>3</sub> ) <sub>0.97</sub> (OH) <sub>1.38</sub>	175.12	2.37	1.35	0.39	3.79	0.99	9.61	0.97	17.17
PC-12	C <sub>9</sub> H <sub>5.05</sub> O <sub>0.68</sub> (OCH <sub>3</sub> ) <sub>0.95</sub> (OH) <sub>1.31</sub>	175.65	2.35	1.34	0.44	4.26	0.87	8.42	0.95	16.77
PC-13	C <sub>9</sub> H <sub>4.94</sub> O <sub>0.80</sub> (OCH <sub>3</sub> ) <sub>0.94</sub> (OH) <sub>1.17</sub>	174.77	2.35	1.34	0.49	4.76	0.68	6.61	0.94	16.67
PC-14	C <sub>9</sub> H <sub>5.34</sub> O <sub>1.12</sub> (OCH <sub>3</sub> ) <sub>0.88</sub> (OH) <sub>1.12</sub>	174.86	2.32	1.32	- <sup>a</sup>	- <sup>a</sup>	- <sup>a</sup>	- <sup>a</sup>	0.88	15.60
PC-15	C <sub>9</sub> H <sub>4.74</sub> O <sub>0.95</sub> (OCH <sub>3</sub> ) <sub>0.86</sub> (OH) <sub>1.14</sub>	173.69	2.32	1.34	- <sup>a</sup>	- <sup>a</sup>	- <sup>a</sup>	- <sup>a</sup>	0.86	15.35

<sup>a</sup>These contents were not determined due to poor resolution(overlap of signals for aromatic and aliphatic hydroxyl groups).

Signal assignments,  $\delta$ -Value(ppm)

- A:  $\delta = 2.0$ , aliphatic acetoxy protons
- B:  $\delta = 2.3$ , aromatic acetoxy protons
- C:  $\delta = 3.75, 3.80$ , methoxyl protons
- D:  $\delta = 4.1-6.2$ , aliphatic protons
- E:  $\delta = 6.9-7.1$ , aromatic protons

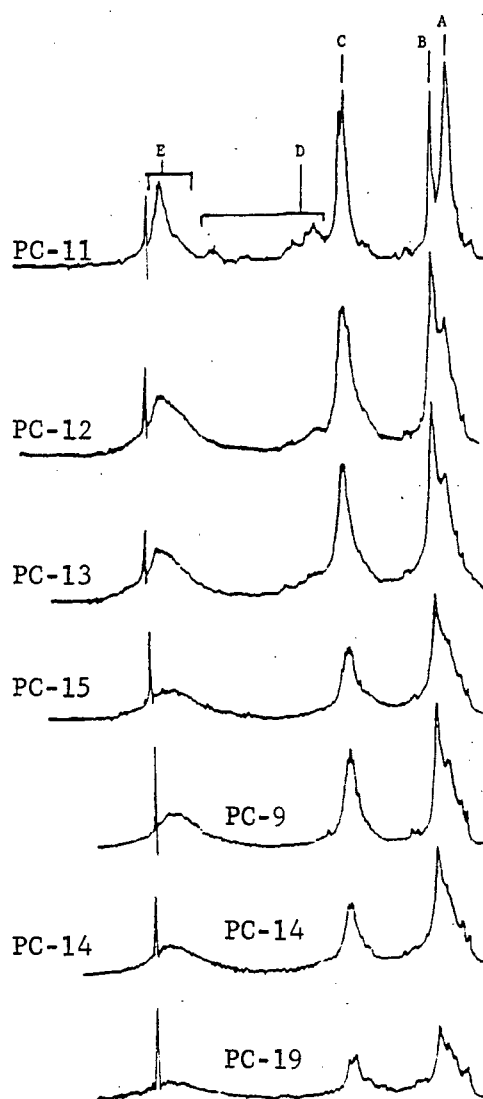


Figure 20. Differential NMR spectra of cooking time and acid concentration series.

Fig. 20 shows the comparison of NMR spectra for the two series of samples, i.e. cooking time series (PC-11 through PC-15) and acid concentration series (PC-9, PC-14 and PC-19). Signal broadening, which is obvious from the appearance of the spectra is thought to be due to a tendency toward rigidity caused by cross linking in the molecular structure of lignin (107). Lignin obtained from the short cook (PC-11) gave more sharply defined spectrum than those from long cooks (PC-12 through PC-15 and PC-19). The reason for this is the fact that sample PC-11 has relatively low molecular weight, which makes for greater mobility of the molecules in solution. The variation of molecular weight of those two series of lignin samples will be extensively discussed later in connection with the GPC analysis.

The spectra shown in Fig. 20 also demonstrate that the size of aliphatic acetoxy signals at  $\delta$  2.00 decreased markedly as cooking time increased. The intensity of the signal for the aliphatic acetoxy protons of the acetylated lignin should correspond to three times of that of aliphatic hydroxyl protons of the parent lignin. The significant collapse of aliphatic hydroxyl groups in longer cooks can be explained by the fact that these hydroxyl groups were used for carbon-carbon linkages to form self-condensation products (104). It was noted that high aliphatic hydroxyl group content in the parent lignin was associated with the low molecular weights of the acetylated lignin. This observation will be discussed in more detail later.

There was no noticeable signal around  $\delta$  6.1, indicating the absence of benzylic hydroxyl groups in the parent lignin samples, despite the fact that benzyl ether content in MWL was reported to be more than 20 per 100 C<sub>9</sub>-units (2,67). Benzyl alcohol in acidic medium is so unstable (120) that almost complete recondensation reaction involving ben-

zyl alcohol seems to take place (108) during the organosolv cooking and as a result, there were no longer free benzylic hydroxyl groups to be acetylated.

Table 11 shows a much higher content of aromatic hydroxyl group in the parent lignins than the reported values, which range 20-25 per 100 C<sub>9</sub>-units. This can be explained by the release of phenolic hydroxyl group due to almost complete splitting of the alkyl-aryl ether bonds of lignin during acid-catalyzed cooking (98). Another reason for this is the fact that the functional group contents determined by NMR spectra are usually higher than those determined by other methods because of the overlap of signals from different types of protons. This comparison will be discussed later with the results of phenolic hydroxyl determination by the spectrophotometric method.

The estimated methoxyl contents range 86-97 per 100 C<sub>9</sub>-units. Table 11 and Fig. 20 show that methoxyl group content of the isolated parent lignins decreases significantly as cooking time increases from 5 to 20 minutes, or as acid concentration increases from 0.025 to 0.1 N HCl. The latter confirms the dependency of methoxyl content on acidity of cooking medium (38). In general, the methoxyl contents determined by NMR spectra were higher than those determined by the TAPPI method (Table 8), as to the case of phenolic hydroxyl content, and this general trend is in good agreement with previous results (57).

Although the change seems to be very small, the content of aromatic protons was found to decrease slightly as cooking time increased as indicated in Table 11. This finding has a very significant meaning. Table 11 shows that the content of aromatic protons for short cook (PC-11) is 2.37 per C<sub>9</sub>-unit, far smaller value than 3 protons per guaiacyl nucleus. This means, if there were lit-



tle or no syringyl units in the Douglas-fir organosolv lignin molecules, about 63% of the aromatic rings in the lignin molecules must be somewhat condensed form and have only two aromatic protons per  $C_9$ -unit, while about 37% of aromatic rings are non-condensed and have three hydrogen atoms on each aromatic ring of the lignin molecule. The content of aromatic proton for long cook (PC-15) was found to be 2.32 per  $C_9$ -unit (Table 11), indicating 68% of aromatic rings are condensed and 32% are non-condensed. The results signify the finding that more condensed lignins were isolated as cooking time increased.

Aliphatic protons attached to the side-chains of lignin molecules were difficult to estimate due to overlapping and interference from the strong methoxyl proton signal as mentioned before. As a result, accurate account for the aliphatic protons was not possible. However, relatively low content of these protons was believed to be due to an elimination reaction of  $\alpha$ - and  $\beta$ -protons in the cyclic  $\alpha$ -aryl ethers by acid hydrolysis (98,106).

Methylene protons, terminal methyl protons and possibly some highly shielded aliphatic protons, which are not attached directly to oxygen functions, were excluded from the calculations because they gave insignificant signals and could hardly be distinguished from the baseline noise.

There was no evidence for the presence of any contamination from carbohydrates in the acetylated organosolv lignin samples used for NMR spectra. None of the lignin spectra shows any detectible signals in the  $\delta$  8-11 range, indicating none or very low content, if any, of carboxylic or aldehyde protons.

### 5.7.2 Infrared spectra

Infrared spectra obtained from two shortened series of reduced lignin samples, cooking time series (Fig. 21) and acid concentration series (Fig. 22) were analyzed, and the results were compared with those obtained from NMR spectra of the same samples. The test was designed to confirm the structural changes of lignin molecules which may have occurred as the results of the various cooking conditions. Since a general similarity of IR spectra in Figs. 21 and 22 is evident for all the samples, only those bands which varied markedly are discussed. The absorption band assignments presented in Table 12, based on earlier investigations (23, 86, 121, 146, 147) give a considerable degree of confidence in most cases except in the region  $1400-1000\text{ cm}^{-1}$ , where various aromatic ring vibration modes and C-O stretching modes occur (86).

As with many other polymers, the complexity of lignin molecules causes band overlapping, diffuse bands, and cross-linking may dampen vibrations (23). Nevertheless, as evident in Figs. 21 and 22, structural changes in the lignin molecules due to the different cooking conditions can be observed. In general, samples collected after short cooking gave sharper absorption bands in their IR spectra upon reduction. On the hand, an increase in the acid concentration of the cooking liquor did not change the absorption bands markedly.

Broad bands at  $3440-3460\text{ cm}^{-1}$  shown in Figs. 21 and 22 are due to O-H stretching vibration and medium or weak bands at  $1390\text{ cm}^{-1}$  seem to be due to O-H bending vibration. Changes of these bands in

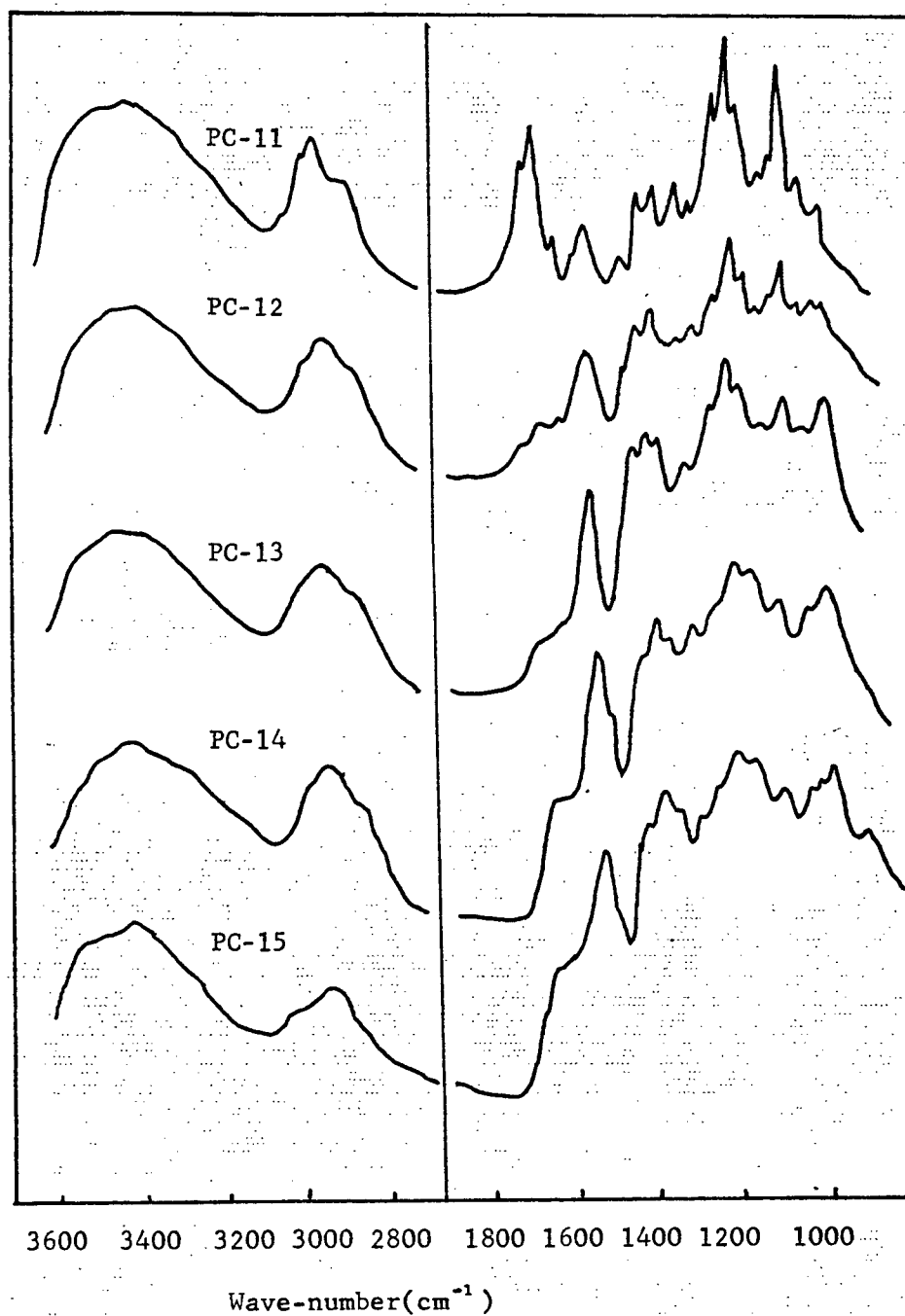


Figure 21. Effect of cooking time on IR spectra of reduced Douglas-fir lignin samples.

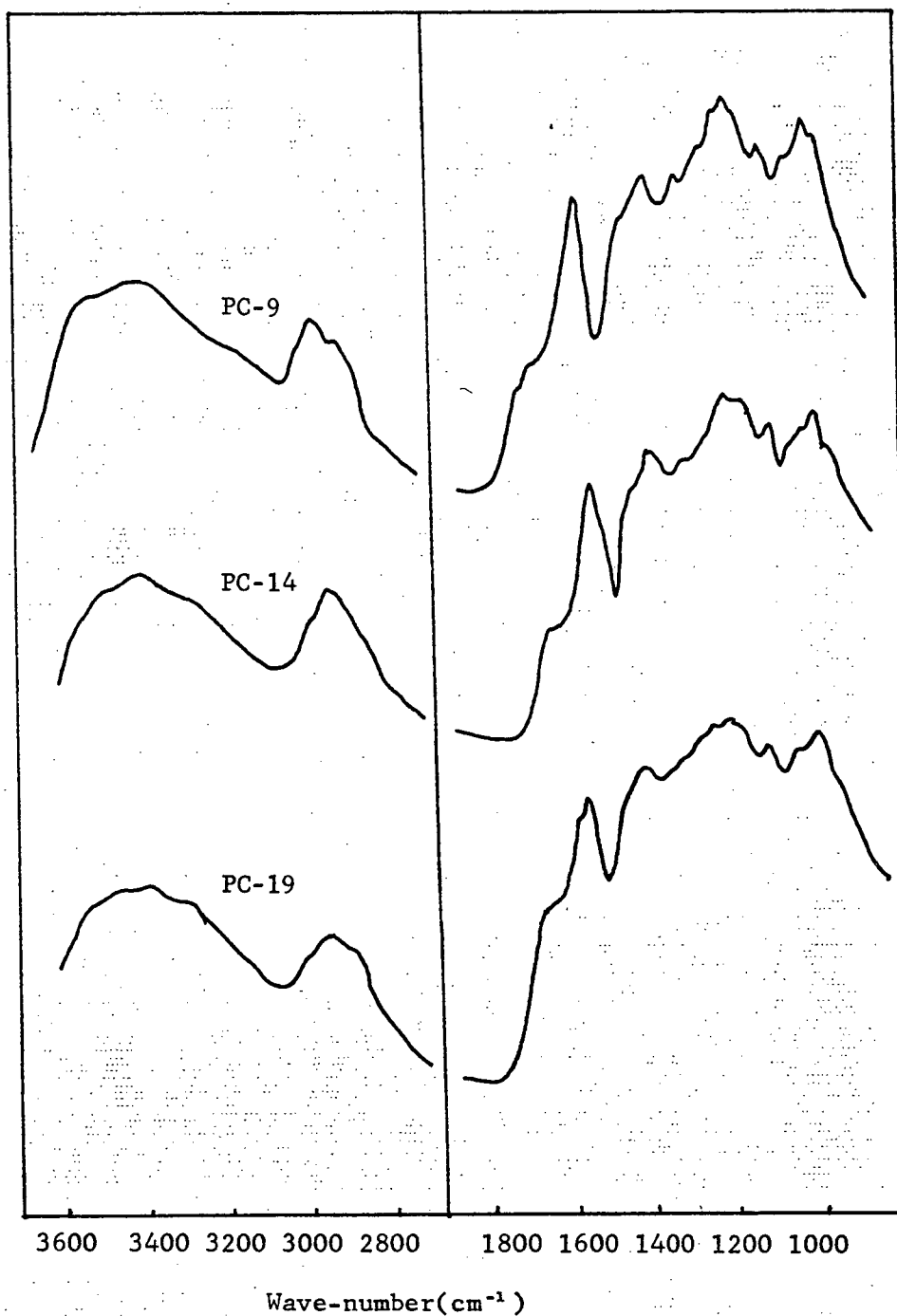


Figure 22. Effect of acid concentration on IR spectra of reduced Douglas-fir lignin samples.

Table 12. Assignments of absorption bands in IR spectra of reduced Douglas-fir lignin samples.

Wave-number( $\text{cm}^{-1}$ )	Assignments
3440-3460	O-H stretching vibration
2990, 2940, 2850	C-H stretching vibration
1710, 1690, 1640	C=O stretching vibration
1560	aromatic skeletal vibration
1470	C-H deformation vibration
1430	C-H bending vibration/aromatic skeletal vibration
1390	O-H bending vibration(?)
1300	condenced guaiacyl(?)
1260	uncondenced guaiacyl
1230, 1190	asymmetric stretching vibration of aryl-alkyl ether(?)
1100	uncondenced guaiacyl(?)
1010	condenced guaiacyl(?)
900	C-H out-of-plane bending vibration

various spectra were found to be insignificant, indicating that the total hydroxyl (aromatic and aliphatic) content changes very little with different cooking conditions.

Bands at  $2990\text{--}2850\text{ cm}^{-1}$  are characteristic of various types of C-H bonds. No significant effect of cooking conditions on the intensity of these bands was noted, though sample PC-11 (5 min; 0.025 NHC1) shows little sharp bands.

Since the lignin samples were reduced with sodium borohydride, absorption bands which are usually well defined ones for carbonyl group seem to disappear at  $1735\text{ cm}^{-1}$  and  $1375\text{ cm}^{-1}$  (146). The IR spectrum of the sample PC-11 shows a strong peak at  $1690\text{ cm}^{-1}$  while spectra for the other samples show only a trace or very weak peaks at this wave number (Fig. 21). Normally, this is very difficult to explain because both unconjugated ketones and conjugated acid or esters absorb at  $1715\text{ cm}^{-1}$ , which can be shifted to  $1690\text{ cm}^{-1}$  (146). However, a possible explanation for this observation can be found because of the fact that the intensity of the band decreases gradually as cooking time increases. As mentioned earlier in NMR spectroscopic analysis, there was no evidence for any aldehyde protons or carboxylic protons in the acetylated lignin samples including PC-11. Therefore, the strong band at  $1690\text{ cm}^{-1}$  in the IR spectrum of PC-11 is thought to be originated from unconjugated ketone and seems to be attributable to the presence of impurities (26,40,86), which can be affected by cooking time. The most probable impurities with unconjugated ketones are flavones, such as dihydroquercetin (XXXI), which can cause a shift of about  $60\text{ cm}^{-1}$  to the longer wave-length due to O-hydroxyl group chelated to the carbonyl group (26,86).

Dihydroquercetin (XXXI), which has been identified as major flavanone of Douglas-fir (33,141), can not be completely removed by alcohol-benzene extraction as mentioned earlier, and thus is believed to be liberated from sawdust together with lignin during organosolv cooking. Because of low yield (9.12%) of the isolated lignin (FRACTION II) from short cook (PC-11; 5 min), the relative concentration of this impurity is high, while the intensity of the peak for this impurity in the sample from long cook (PC-15; 20 min) is almost unnoticed due to high yield (29.12%) of the isolated lignin sample (Table 3). As a result, the strong peak at  $1690\text{ cm}^{-1}$  decreases as cooking time increases.

Probably, the most significant observation from Fig. 21 is that the intensities of the bands at  $1260\text{ cm}^{-1}$  and  $1100\text{ cm}^{-1}$  for uncondensed guaiacyl nucleus decrease substantially as cooking time increases, confirming the results obtained from NMR spectra. Analysis of NMR spectra indicated that non-condensed aromatic nuclei were 37% for short cook sample PC-11 (5 min) and those for long cook sample PC-15 (20 min) were 32% (Table 11). This observation is the most important evidence for higher molecular weights of samples from longer cooks. This will be discussed later in connection with GPC analysis.

A somewhat smaller decrease in longer cooks was also noted for the band at  $1230\text{ cm}^{-1}$  (Fig. 21), which has been assigned to asymmetric C-O vibrations of aryl-alkyl ethers. The recondensation reaction, which results in high molecular weights of lignin samples from longer cooks, must have followed the initial scission of aryl-alkyl ether linkages due to the prolonged cooking. This evidence was also confirmed by NMR analysis.

Methyl and methylene groups, and ethylenic double bond were not analyzed due to the lack of supporting evidences in NMR spectra. Estimation of methoxyl content by IR spectra (80,147) was not attempted because of poor resolution between wave-number  $1200-1470\text{ cm}^{-1}$ , and broadness and overlapping of the absorption bands as mentioned earlier.

The IR spectra of unreduced lignin samples may be more informative as to the original conditions. However, unreduced samples were observed to be less stable since a continued color change was evident on standing at room temperature.

### 5.7.3 Ultraviolet spectra

A series of reduced lignin samples were analyzed using UV spectrophotometer to examine the effect of cooking time on the content of phenolic hydroxyl group. Fig. 23 shows that all five samples have somewhat similar UV spectra. The maxima appeared around 294 to 302 nm of the difference curve which is characteristic for only the phenolic hydroxyl group (11,12,77,131). The method used in the present study is based upon the characteristic UV absorption of phenols in alkaline solution. According to Aulin-Erdtman (11,12), the bathochromic shift of the characteristic 280 nm absorption maximum of lignin in alkaline solution takes place due to the ionization of the phenolic group.

In the present method, UV difference curves were obtained directly by scanning the alkaline vs. the neutral lignin solutions, respectively placed in the sample and reference cells of the spectrophotometer (77). The results shown in Table 13 indicate that the



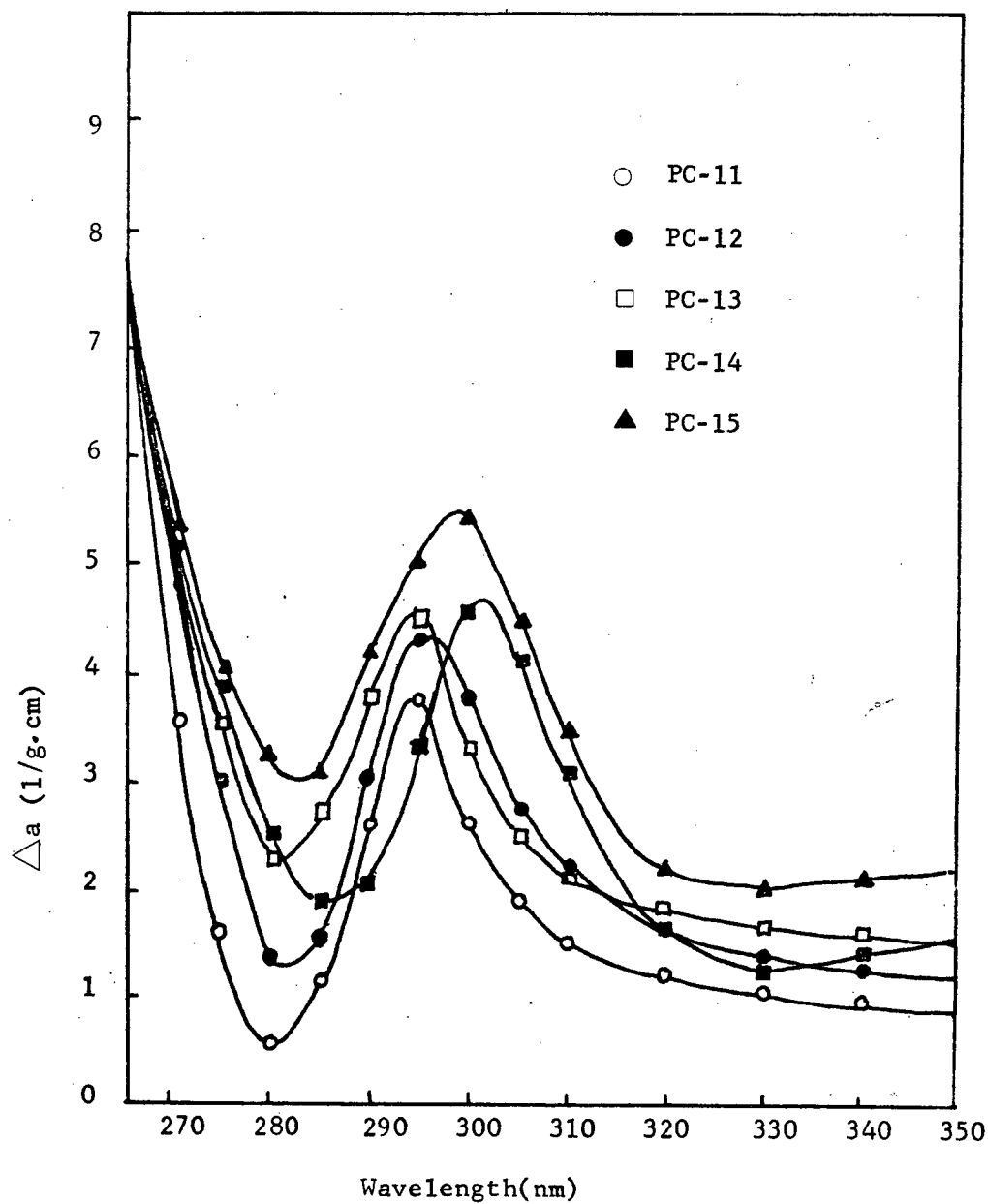


Figure 23. Difference UV spectra of reduced lignin samples.

Table 13. Spectrophotometric determination of phenolic hydroxyl group in Douglas-fir lignin.

Sample No.	$\lambda_{\text{max.}}$ (nm)	Absorbance	$\Delta a_{\text{max.}}$ (1/g·cm)	Phenolic OH (%)
PC-11	294	0.38	4.75	1.97
PC-12	296	0.44	5.50	2.28
PC-13	294	0.46	5.75	2.39
PC-14	299	0.55	6.88	2.86
PC-15	302	0.47	5.88	2.44

phenolic hydroxyl group content increased as cooking time increased (up to 17 min) and then slightly decreased, confirming the similar results of NMR spectra analysis. The calculated phenolic hydroxyl content of this series ranged from 1.97 to 2.86% (based on total weight of lignin) according to the equation provided by Goldschmid (77). This content based on lignin weight is equivalent to 0.20 to 0.29 hydroxyl groups per  $C_9$ -unit, if converted by using the apparent unit weights listed in Table 11. This content is slightly lower than the recorded phenolic hydroxyl contents (0.27-0.29/ $C_9$ -unit) of several softwood MWL (148).

Considering these results in relation to those obtained from NMR spectra, it seems likely that mainly the active aromatic hydrogens, rather than phenolic hydroxyl groups, are involved in the recondensation reaction of lignins to form C-C linkages because the content of aromatic hydrogen was found to decrease as the cooking time increased, whereas the phenolic hydroxyl group content actually increased. It is significant that the net hydroxyl content increased gradually due to a possible cleavage of the aryl-alkyl ether linkages as cooking time increased, even though some of the phenolic hydroxyl groups might have been consumed in the recondensation reaction.

As mentioned above, the phenolic hydroxyl content, determined by the spectrophotometric method, was lower than that obtained from NMR spectra (Table 11). There is one serious limitation in the method used for this test. Because of the assumption that every phenyl propane unit of lignin carries one methoxyl group, this method can not be applicable to those lignin samples which contain syringyl nuclei (77).

## 5.8 Macromolecular Analysis of Isolated Organosolv Lignin

### 5.8.1 Gel permeation chromatographs

#### 5.8.1.1 Effect of cooking time on molecular weight

According to Goring (81), during the chemical delignification process, penetration by cooking liquor into the secondary wall occurs initially and the low molecular weight lignin of the secondary cell wall is the first to be extracted. As cooking time increased, penetration by the cooking liquor into the middle lamella occurred and lignin of high molecular weight was extracted. Thereby, both  $\bar{M}_w$  and  $\bar{M}_n$  changed as cooking time increased as shown in Table 14.  $\bar{M}_w$  and  $\bar{M}_n$  first increased up to about 12 minutes and then decreased as cooking period further increased up to 20 minutes (Fig. 24).

Lora and Wayman (104) suggested earlier that there are two reactions involved during the delignification under acidic conditions. The faster first reaction, primarily occurring by breaking of lignin-carbohydrate bonds, produces soluble lignin fractions and the slow second reaction, which is essentially a condensation, results in insoluble lignin fractions in the presence of the organic acids (acetic acid and formic acid) formed during the autohydrolysis. The results shown in Table 14 clearly demonstrate that at the beginning of cooking, the rate of delignification was fast due to rapid increase of the temperature of the cooking mixture (94,96), and resulted in low molecular weight lignin fractions. However, as cooking time increased, a slow recondensation starts to produce higher molecular weight lignin fractions.

Table 14. Molecular weight averages and polydispersity indices of acetylated Douglas-fir lignin samples.

Sample No.	Cooking time(min.)	Acid(HCl) concentration(N)	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
PC-9	17	0.025	7,675	996	7.71
PC-11	5	0.050	7,984	1,092	7.31
PC-12	9	0.050	9,821	1,127	8.73
PC-13	12	0.050	10,122	1,144	8.85
PC-14	17	0.050	9,293	1,002	9.27
PC-15	20	0.050	8,762	823	10.65
PC-19	17	0.100	10,186	1,005	10.13

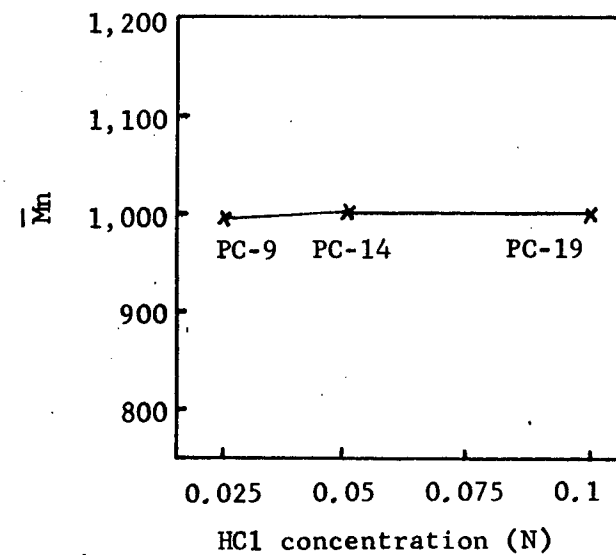
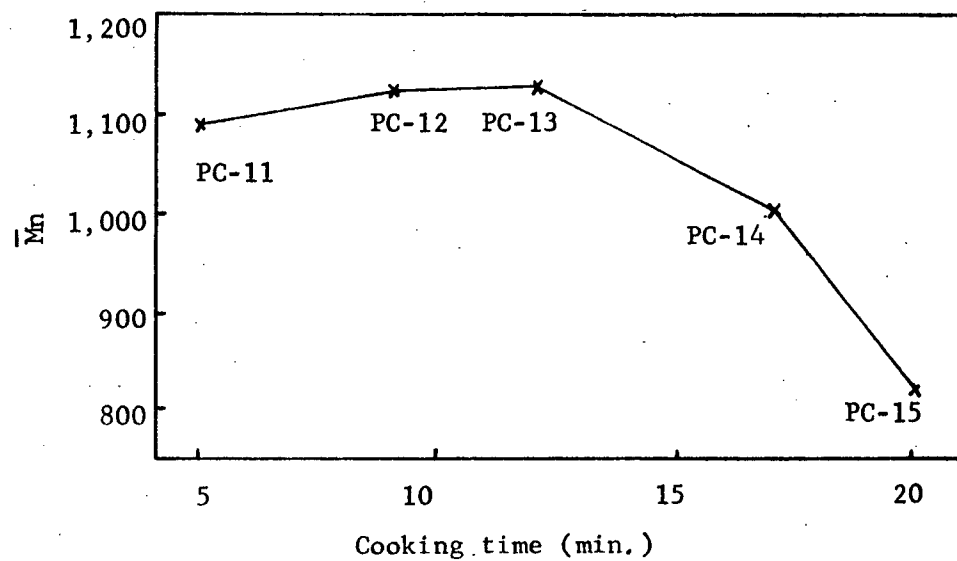
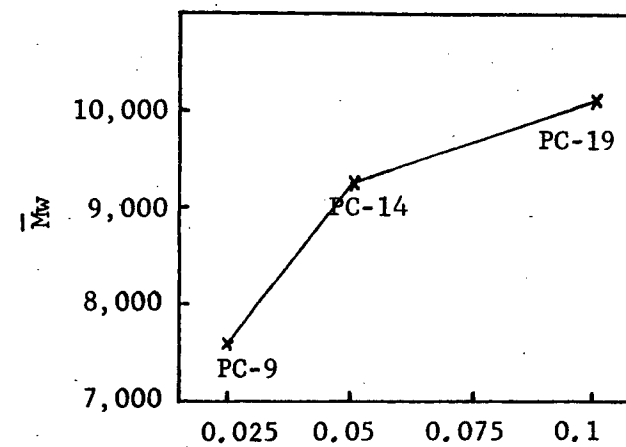
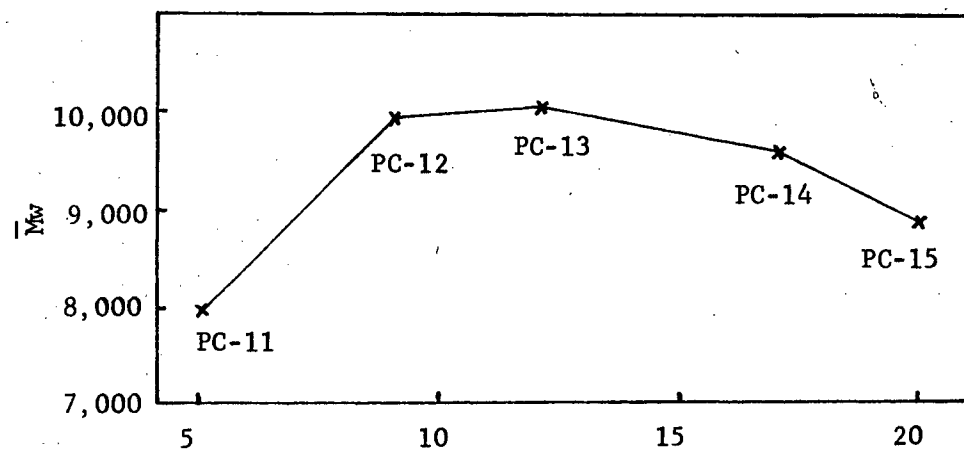


Figure 24. Effect of cooking conditions on molecular weight averages of acetylated lignin samples.

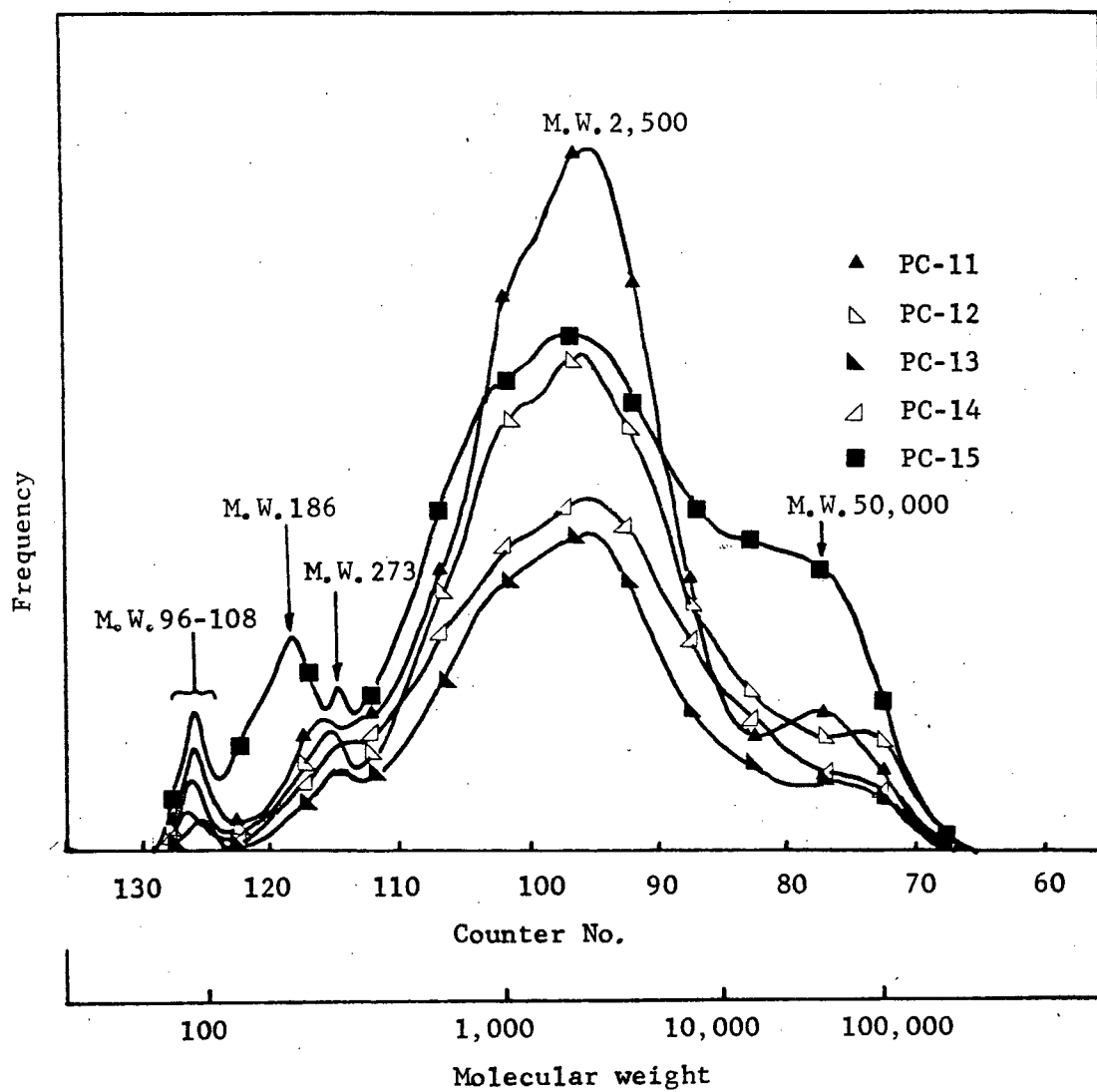


Figure 25. Change in molecular weight distribution with cooking time.

tions (104). By the time (around 12 min) the temperature reaches 200°C, however, the extracted lignin in the cooking liquor is subject to degradation due to acidic condition (0.05 N HCl) as cooking time is prolonged. This results in a decrease of  $\bar{M}_w$  and  $\bar{M}_n$  as shown in Table 14 and Fig. 24. Fig. 25 suggests that there is no substantial change in the predominant fraction (main peak around molecular weight 2,500) of the acetylated lignin samples even if cooking time is changed to 20 minutes. A short cook (PC-11; 5 min) gives a narrow main peak while longer cooks (PC-12 through PC-15; 9-20 min) give broader peaks which are more or less symmetrical in shape. Similar distributions were also reported in earlier studies (35, 89).

In the MWD curve (Fig. 25) of the longest cook (PC-15; 20 min), it can be seen that a very distinct fraction ranged between 10,000 and 50,000. This observation may be explained by the self-condensation of lignin through C-C linkages due to carbonium ions formed in different positions on the side-chain of the phenyl propane units under acidic conditions during prolonged periods of cooking time (35, 165). Somewhat similar high molecular weight fractions were also found in the other samples (Fig. 25). The unusually low contents of hydrogen and oxygen in these lignin samples, as mentioned in NMR spectral analysis, may be the direct result of the formation of new C-C linkages. NMR spectra in Fig. 20 clearly illustrate that the sharp singlet signal for aliphatic hydroxyl groups ( $\delta$  2.0) collapses significantly as cooking time increases.

There is another explanation for the high molecular weight lignin fractions. Sarkanen *et al.* (151), in their study on hardwood lignins from organosolv cooking (temperature=135-165°C; catalyst=aluminum chloride; cooking time=1-6 hrs), found that organosolv lignins have a



tendency to form high molecular weight associated complexes even under quite strongly alkaline aqueous conditions. Even though there is no evidence for this tendency in the present test, it is quite possible that high molecular weight associated fractions might have formed in the acetylated samples used for GPC analysis.

Another significant observation is the small, but very distinct peaks around molecular weight 96-108 (Fig. 25). Especially, sample PC-15 clearly shows three peaks in its MWD curve around molecular weights of 108, 186 and 273, which can be interpreted as monomer, dimer and trimer of the lignin guaiacyl unit, respectively. These peaks seem to represent the degradation products formed during the cooking period. Recently, Sarkanen et al. (150) noted five or six peaks in the lower molecular weight region in the MWD curve of organosolv lignin from cottonwood by Sephadex G-50. Smaller fractions than tetramer were reported to be identified by gas chromatography by Dimmel et al. (49), in their study on molecular weight changes in lignin during anthraquinone-alkali pulping. Table 24 also shows that polydispersity index ( $\bar{M}_w/\bar{M}_n$ ) increases as cooking time increases, rising from 7.31 for a 5-min cook (PC-11) to 10.65 for a 20-min cook (PC-15). Such high values of polydispersity, indicating a wide range of MWD, are not unusual for these lignin samples because of simultaneous formation of low molecular weight degradation products and high molecular weight recondensation products during cooking as mentioned before.

#### 5.8.1.2 Effect of acid concentration on molecular weights

Košiková and Polčín (97) reported that delignification with hydrochloric acid in aqueous organic solvent is basically an

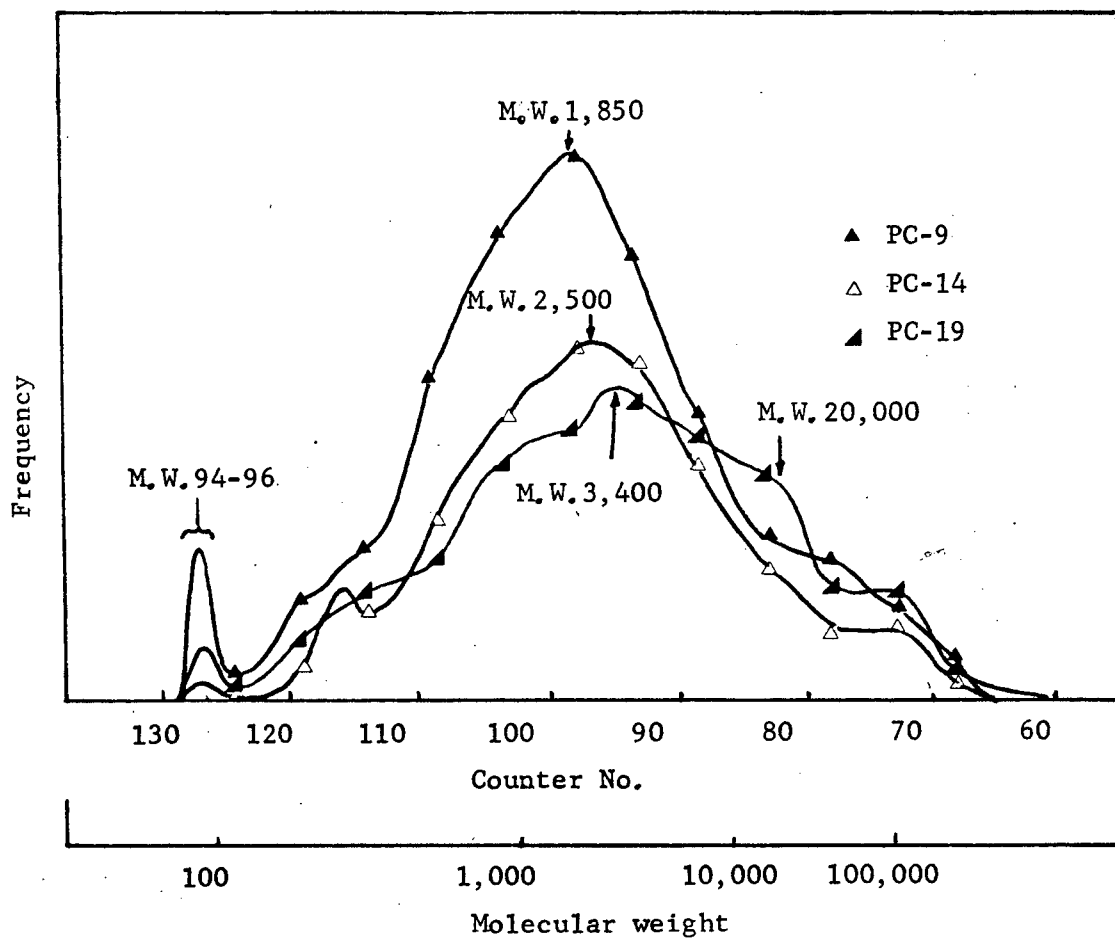


Figure 26. Change in molecular weight distribution with acid concentration.

acidolytic splitting of the lignin macromolecules and of the lignin-carbohydrate complex into individual components soluble in the cooking liquor. As indicated in Table 14, and Fig. 24,  $\bar{M}_w$  shows a large increase while  $\bar{M}_n$  shows a moderate increase as acid concentration increased from 0.025 to 0.1 N.

The MWD curve for sample PC-19 (0.1 N HCl; 17 min) shows a new peak around molecular weight 20,000. This high molecular weight fraction may be the products of the lignin recondensation reaction or associated complexes, as mentioned before. The balance between decomposition and recondensation is evidently one of the deciding factors for MWD of low and high fractions of the isolated organosolv lignin, and the two may be taken as results of two competing reactions.

One of the most interesting observations in Fig. 25 is the shift of the predominant peak toward the high molecular weight end of the MWD curve as acid concentration increased. The main peaks of samples PC-9 (0.025 N HCl), PC-14 (0.05 N HCl) and PC-19 (0.1 N HCl) appear at molecular weights of 1,850, 2,500 and 3,400, respectively. The shift of the main peak, which represents the predominant fraction of the isolated lignin, toward the higher molecular weight end seems to be the main reason for the increase of  $\bar{M}_w$  and  $\bar{M}_n$  as acid concentration increased.

The increase of the polydispersity index from 7.71 to 10.13 is also observed as acid concentration increased from 0.025 to 0.1 N (Table 14). Preliminary cooking experiment with the same acidic condition (0.025 N HCl) in methanol-water (7:3 by volume) as cooking liquor, resulted in a much lower polydispersity index (3.68), indicating that not only acid concentration, but also solvent power affects the polydispersity of molecular weight of the isolated organo-

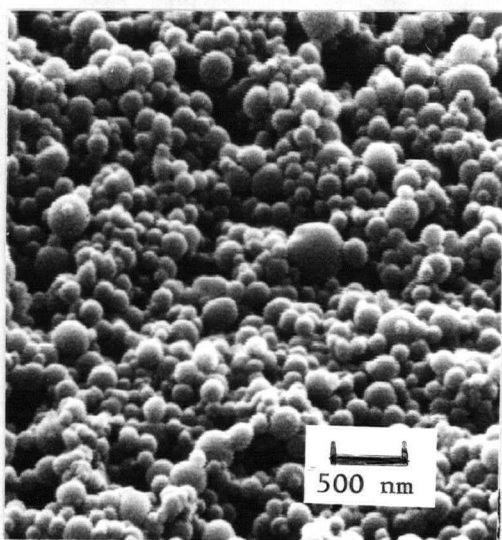
solv lignins. These results prove that acetone-water is a better solvent system for rapid delignification in organosolv pulping.

#### 5.8.2 Scanning electron photomicrographs

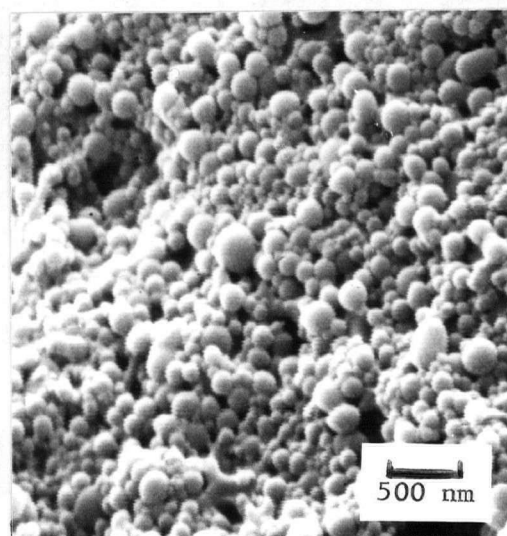
The wide range of lignin particle size can be seen in the scanning electron photomicrographs (Figs. 27 and 28). For example, the spherical particles of sample PC-15, having the highest value of polydispersity index, includes a wide range of particle sizes from smaller than 25 nm up to 500 nm (Figs 27 and 28, and Table 15). The larger particles which seem to be aggregates of the smaller granules (137,138) may be the high molecular weight fractions shown in the MWD curves obtained by GPC.

To correlate MWD curve and particle size distribution (PSD) of the same lignin samples, particle size frequency distribution diagrams based on cooking time (Fig. 29) and acid concentration (Fig. 30) were constructed. Similar patterns of distribution of MWD curve and PSD were found for both the cooking time series (Figs. 25 and 29) and the acid concentration series (Figs. 26 and 30). There is no hard evidence, however, as to whether there is a direct relationship between the MWD curves obtained from GPC and PSD of the same lignin samples.

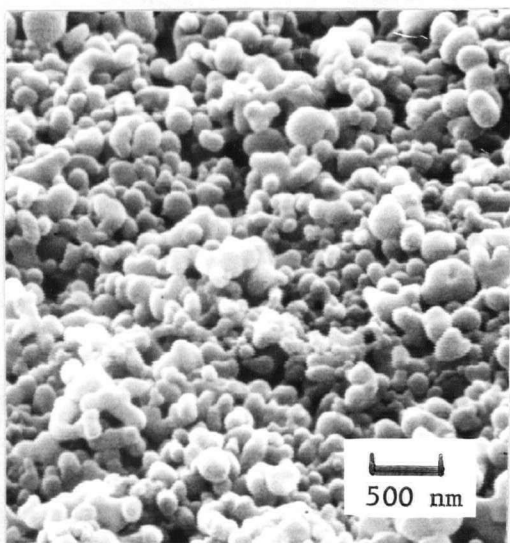
In all SEM photos, there is no evidence of carbohydrate contamination of the acetylated organosolv lignin samples, thus confirming the results of NMR spectra analysis on the same lignin samples.



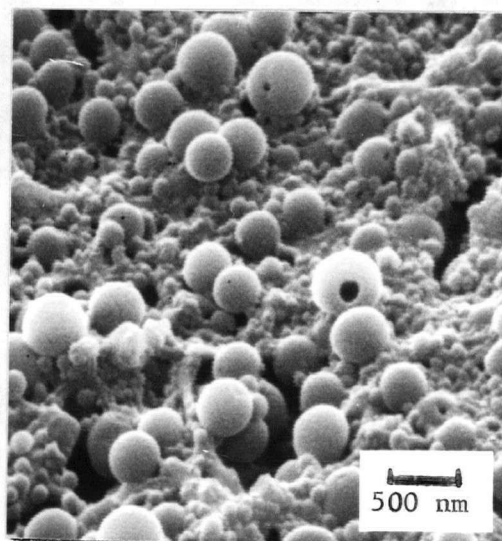
PC-11 (5 min)



PC-12 (9 min)

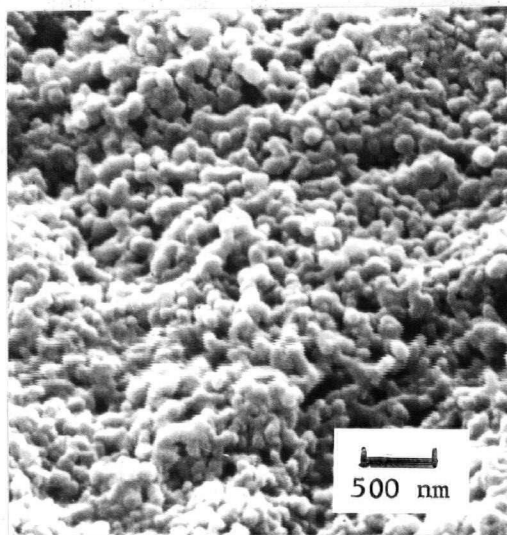


PC-13 (12 min)

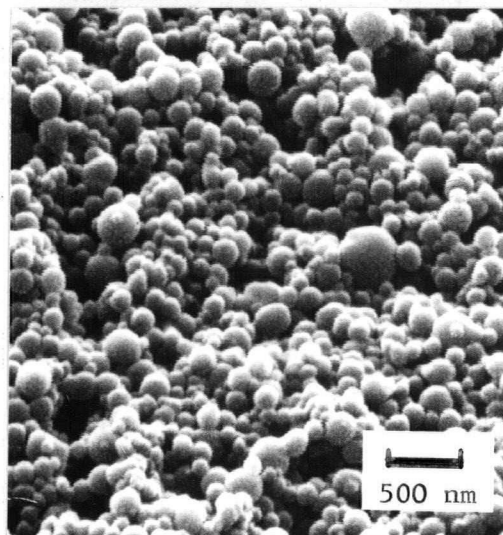


PC-15 (20 min)

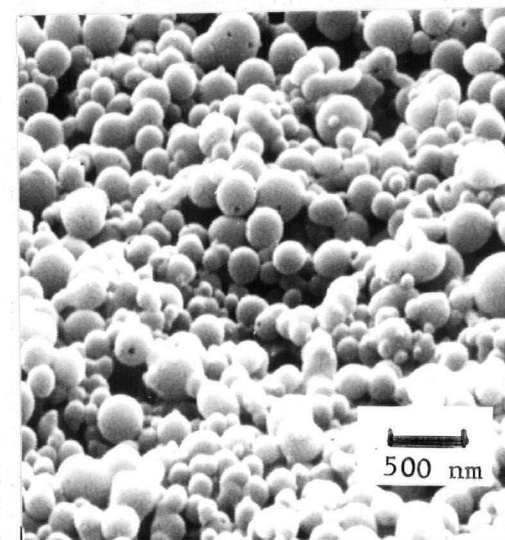
Figure 27. Scanning electron photomicrographs of particle size variation with cooking time.



PC-9 (0.025N HCl)



PC-14 (0.05N HCl)



PC-19 (0.1N HCl)

Figure 28. Scanning electron photomicrographs of particle size variation with acid concentration.

Table 15. Frequency of particle sizes of acetylated lignin samples.  
(%)

Sample No. Diameter of particle (nm)	PC-11	PC-12	PC-13	PC-14	PC-15	PC-9	PC-19
< 25	5.2	3.5	4.2	3.4	8.1	2.3	-
25 — 50	21.5	12.8	10.4	10.3	26.8	11.1	-
50 — 75	34.8	27.9	16.2	16.2	30.9	46.9	2.6
75 — 100	32.6	33.3	26.2	20.5	12.1	33.4	7.9
100 — 125	4.1	10.9	24.2	27.4	3.4	3.6	28.3
125 — 150	1.1	3.8	11.2	15.0	2.0	2.0	31.6
150 — 175	-	4.5	1.9	3.4	1.3	-	9.2
175 — 200	0.3	1.9	3.1	0.9	-	0.7	4.6
200 — 225	0.3	-	-	0.4	2.0	-	6.6
225 — 250	-	0.3	0.8	1.3	0.7	-	5.3
250 — 275	-	0.3	0.8	-	1.3	-	0.7
275 — 300	-	-	-	0.4	-	-	2.0
300 — 325	0.3	-	0.4	0.4	0.7	-	-
325 — 350	-	0.6	0.8	-	4.7	-	-
350 — 375	-	-	-	0.4	0.7	-	0.7
375 — 400	-	-	-	-	0.7	-	-
400 — 425	-	-	-	-	-	-	-
425 — 450	-	-	-	-	2.0	-	0.7
450 — 475	-	-	-	-	-	-	-
475 — 500	-	-	-	-	1.3	-	-

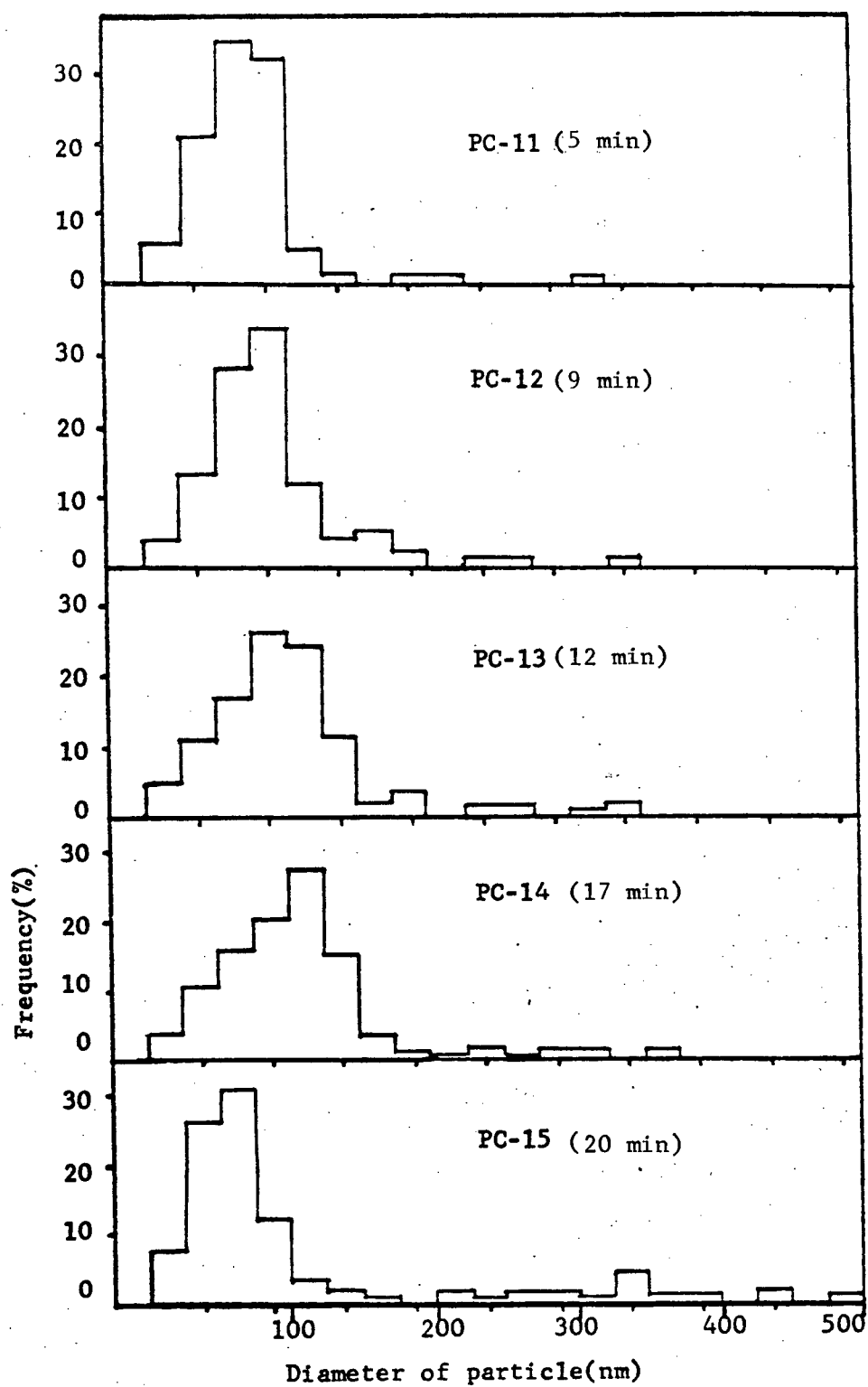


Figure 29. Effect of cooking time on particle size frequency distribution.



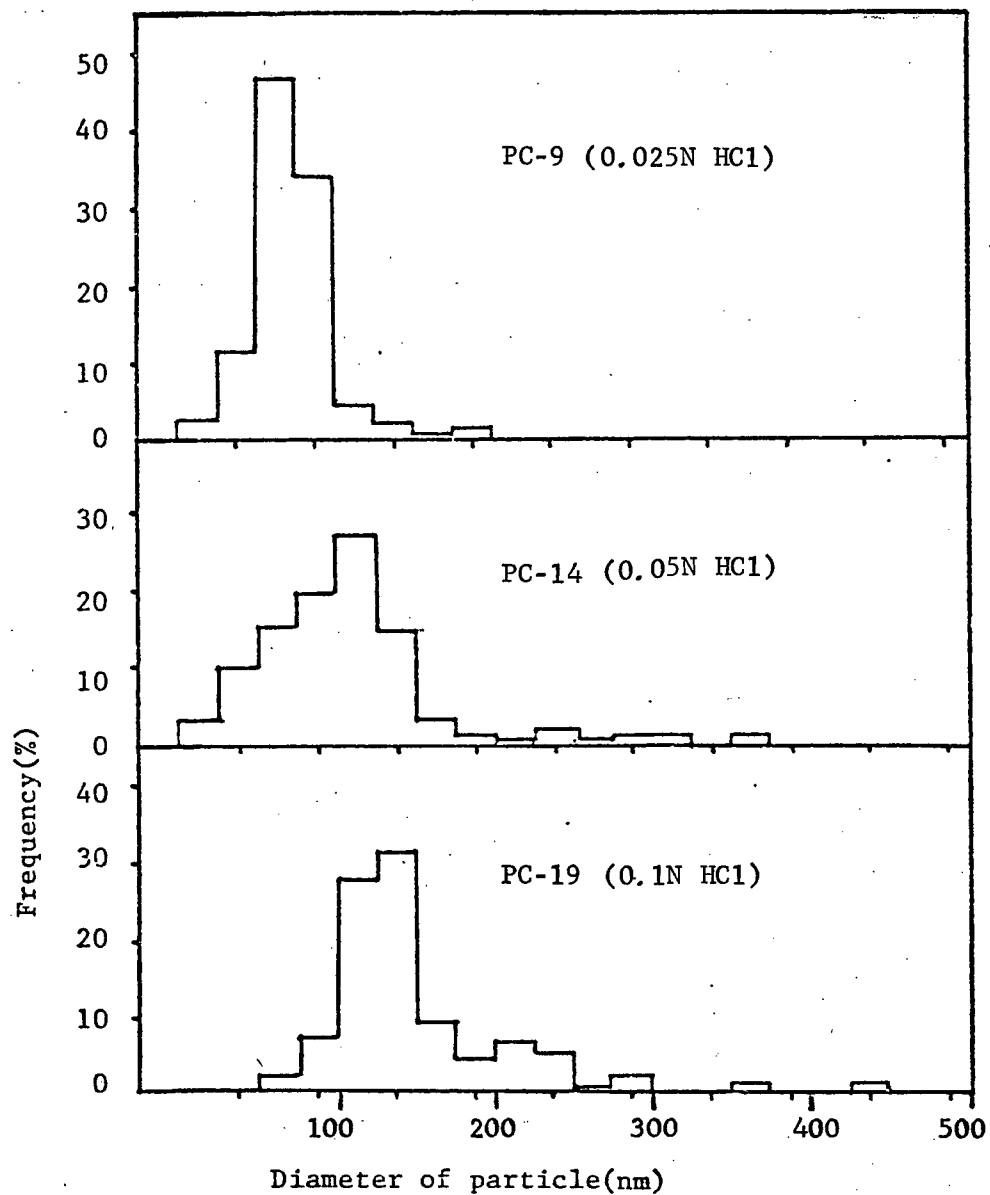


Figure 30. Effect of acid catalyst concentration on particle size frequency distribution.

## 6. RECOMMENDATIONS

The information derived in this study on the isolation and characterization of Douglas-fir organosolv lignin from the acid-catalyzed cooking allows recommendations for further research on organosolv lignin.

1. An improvement of the isolation technique for the water-soluble lignin fraction, which seems to have very low molecular weight and high content of phenolic hydroxyl group, is required. Such water-soluble lignins, isolated in large amounts, could have commercial importance.

2. Simple and effective separation techniques for dissolved sugars from the spent liquor should be developed. Determination of these sugars could give invaluable information on pulp and the isolated organosolv lignin.

3. Detailed studies should be initiated regarding to competition between the lignin degradation reaction (hydrolysis) and recondensation reaction since the latter limits lignin fragmentation to low molecular weight products during the cooking.

4. Influence of various organic solvents (both polar and non-polar) used as organic component of the cooking liquor on content of functional groups of the isolated lignins should be studied in more detail.

5. Further studies on lignin macromolecular properties by GPC and SEM are required in order to find firm evidence in support of a close relationship between MWD of lignin molecules and PSD of lignin particles.

## 7. CONCLUSION

The following general conclusions can be drawn from this study.

1. At a constant reaction temperature (200°C), both cooking time and concentration of acid catalyst were found to have a profound effect on the rate of hydrolysis of wood constituents during organosolv cooking. These parameters provide the means to maximize yields of lignin and sugars.

2. Cooking time seems to be a more important parameter than acid concentration in regulating the quantity and quality of the isolated organosolv lignins. In general, lignin yield increased with prolonged cooking. Cooking time was found to affect the content of functional groups and molecular weight of the organosolv lignin.

3. The molecular weights of the isolated organosolv lignin seem to be much lower than those of the protolignin in wood. The probable reason for this is thought to be scission of ether linkages of protolignin molecules through acid hydrolysis during cooking. However, prolonged cooking seems to increase the molecular weights due to autocondensation of lignin molecules.

4. Analyses of NMR, IR, and UV spectra of the isolated lignins provide good evidence of acid hydrolysis of aryl-alkyl ether linkages and the effect of autocondensation reaction during organosolv cooking. The balance of degradation and recondensation reactions was found to be the deciding factor for

the size of molecular weight and polydispersity of the isolated organosolv lignins.

5. SEM photographs show that precipitation of the acetylated organosolv lignins from Douglas-fir sawdust occurs in shape of spherical particles with wide variety of size. The particle diameters ranges from somewhat smaller than 25 nm to about 500 nm. PSD obtained from SEM and MWD curves from GPC were found to have similar distribution patterns.

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