

CHARACTERIZATION OF LIGNIN PRECIPITATED FROM THE SODA BLACK LIQUOR OF OIL PALM EMPTY FRUIT BUNCH FIBERS BY VARIOUS MINERAL ACIDS

M.N. Mohamad Ibrahim*, S.B. Chuah

School of Chemical Sciences
Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia

W.D. Wan Rosli

School of Industrial Technology,
Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia

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ABSTRACT

Soda lignin from oil palm empty fruit bunch was directly isolated by various mineral acids *i.e.* sulfuric acid, hydrochloric acid, phosphoric acid and nitric acid at three levels of concentration (20% v/v, 60%v/v and concentrated). A comparison study was performed through physico-chemical properties and structural features using FT-IR, UV, ¹³C-NMR and nitrobenzene oxidation. The FT-IR results showed that there is no significant difference between the main structures of the lignin isolated by various acids. However, low concentration of phosphoric acid is preferable because of its highest yield. The S: V: H ratio of 7-15:6-11:1 as evaluated by the nitrobenzene oxidation procedure suggests that soda lignin can be classified as belonging to either the cereal straw or grass type. The UV results indicate that phosphoric acid consistently gave the highest absorbance value among the four acids tested in this study regardless of its concentration level. The C¹³-FTNMR spectra, suggest that the lignin structure is independent of the type of acid used for precipitation.

1. INTRODUCTION

Oil palm (*Elaeis guineensis*) is a native of West Africa. It was introduced to various parts of the tropics for its oil-producing fruit. In Malaysia, it is estimated that 2.28 million ha of land is being cultivated with oil palm trees¹. Besides producing palm oil, the industry also generates massive amounts of lignocellulosic residues such as trunks, fronds and empty fruit bunches (EFB). Paper grade pulps from these fibres have since been established^{2,3,4}. However the lignin extracted during

Corresponding author e-mail: mnm@usm.my

the pulping process has so far not being investigated for its usefulness. Before its applications can be considered, knowledge of its structural characterization is required; this study presents such an effort.

Lignin is an amorphous polyphenolic material arising from an enzyme-mediated dehydrogenates polymerization of three major phenylpropanoid monomers, which are coniferyl, sinapyl and p-coumaryl alcohol⁵. The lignin structural elements are linked by carbon-carbon and ether bonds to form tri-dimensional network associated with the hemicelluloses polysaccharides inside the cell wall⁶. Lignin is usually insoluble in all solvents and can only be degraded by physical or chemical treatments⁷. During the chemical pulping process at high-temperature and high-pressure, degradation of lignin occurs and dissolves into the spent liquor. The delignification reactions involved the cleavage of non-phenolic β -O-4 linkage, phenolic α -O-4 linkage and releasing from the associated by the polysaccharide^{8,9}.

The structure of lignin obtained from the EFB fiber is more complex than the structure of lignin extracted from wood due to a complex arrangement of syringyl- and guaiacyl- propane units together with p-hydroxy-propane unit in the EFB fiber. The EFB fiber contains about 17.2% lignin, which is relatively low compare to hardwood and softwood materials¹⁰.

The objective of this investigation is to gain a better understanding on the effects of different mineral acids used in recovering soda lignin. EFB lignin was isolated from the soda black liquor by various type of mineral acids *i.e.* sulfuric acid, hydrochloric acid, phosphoric acid and nitric acid at three levels of concentration (concentrated, 60%v/v and 20% v/v). The obtained lignin was characterized both using destructive technique such as nitrobenzene oxidation and non-destructive methods such as infra-red (IR), ultraviolet (UV), nuclear magnetic resonance (FT-NMR) spectroscopy and gel permeation chromatography (GPC).

2. EXPERIMENTAL

2.1 Material

The empty fruit bunch (EFB) raw material used in this study was supplied by Sabutek (M) Sdn. Bhd., a local company specializing in recycling of EFB. The fibers were washed with water prior to pulping.

2.2 Pulping conditions

The EFB fiber was pulped in a 20 L stainless steel rotary digester unit with 25% NaOH (cooking liquor) for 3 hours at a maximum cooking temperature of 170°C, with a cooking liquor to EFB ratio of 10:1.

The pH of the obtained black liquor was 12.45 and its density 1.02 g/ml. The soda lignin was then precipitated from the concentrated black liquor by acidifying it to pH 2 using various mineral acids at three levels of concentration *i.e.* 20%, 60% and concentrated. The acids used

in this study were sulfuric acid, hydrochloric acid, phosphoric acid and nitric acid. The precipitated lignin was then filtered and washed with water that was adjusted to pH 2 using the corresponding acid used in the earlier step. The soda lignin was then dried in a vacuum oven at 55°C for 24 hours prior to further analysis.

2.3 Analysis of lignin

Nitrobenzene oxidation was carried out by adding 50 mg dry soda lignin into a mixture of 7 ml of 2 M NaOH and 4 ml of nitrobenzene in a 15 ml steel autoclave. The autoclave was sealed tightly with a screw cap fitted with Teflon gasket and heated to 165°C for 3 hours in a preheated thermostat oil bath. After the heating period, the autoclave was cooled with ice water. The mixture was then transferred to a liquid-liquid extractor for continuous extraction with chloroform (5 x 20 ml) to remove any nitrobenzene reduction product and excess of nitrobenzene. The oxidation mixture was acidified by concentrated HCl to pH 3-4 and further extracted with chloroform (5 x 15 ml). The solvent from the second chloroform solution was removed by using rotary evaporator at 40°C under reduced pressure to obtain the nitrobenzene oxidation mixture. The mixture was then dissolved into dichloromethane and made it up to 10 ml. This mixture was used as a stock solution for further analysis of the oxidation mixture⁵.

High performance liquid chromatography (HPLC) was used to analyze the nitrobenzene oxidation mixture. 0.2 ml of stock solution was pipetted into 25 ml volumetric flask and made it up with acetonitrile-water (1:2 v/v). The resulting sample solution was filtered through a Millipore membrane (pore size 0.45 μ) to remove high-molecular weight contaminant and the 3,4,5-trimethoxybenzaldehyde was used as an internal standard. About 20 μ l of the filtrate was injected into the HPLC system (Shimadzu) equipped with Hypersil bond C₁₈ column (particle size 5 μ , 25 mm x 4.6 mm i.d.) to determine qualitatively the oxidation products. A mixture of acetonitrile-water (1:8) containing of 1% acetic acid was used as an eluent with a flow rate of 2 ml/min. The eluent was monitored with an UV (ultraviolet) detector at 280 nm⁵.

The IR spectra were recorded on Perkin-Elmer 2000 spectrophotometer for each sample. The KBr pellets were prepared containing 1% fine ground sample. For the UV spectra, a Hitachi spectrophotometer model was used to obtain the results. Prior to the analysis, the samples (5 mg) were dissolved into 10 ml 90% (v/v) dioxane-water (aliquot). 1 ml of aliquot was further diluted into 25 ml by using 50% (v/v) dioxane-water. The sample was then measured its absorbance for the range of 210 nm to 350 nm¹⁰.

The molecular weight distribution of the lignin was determined using gel permeation chromatography (GPC) on a PLgel 5 μ Mixed-D column. Monodisperse polystyrene was used as the standard. 100 μ l of the sample with the concentration of 1 mg/ml was injected into the GPC. The temperature and flow rate of the column was set at 40°C and 1 ml/min respectively.

The C¹³-FTNMR spectra were obtained from a Bruker Avance 300 operating in the FT mode at 300 MHz under total proton decoupled conditions. The spectra were recorded at 40°C from 200 mg lignin dissolved in 1ml DMSO-d₆ after 3,000 scans. A 90° pulse flipping angle, a 26.6 μ s pulse width and a 1.74 s acquisition time were employed.

3. RESULTS AND DISCUSSIONS

The yields of soda lignin precipitated by different mineral acids at various concentrations (v/v) are shown in Table 1.

Table 1: The average yields of lignin were recorded based on g/100 ml black liquor used

| Concentration (v/v) | Average Yield of lignin in g per 100 ml black liquor for 3 trials | | | |
|---------------------|---|-------------------|-----------------|-------------|
| | Sulfuric Acid | Hydrochloric Acid | Phosphoric Acid | Nitric Acid |
| 100% | 1.42±0.02 | 1.37±0.01 | 1.47±0.02 | 1.20±0.03 |
| 60% | 1.44±0.01 | 1.35±0.02 | 1.45±0.01 | 1.22±0.02 |
| 20% | 1.48±0.01 | 1.40±0.01 | 1.51±0.02 | 1.27±0.02 |

It can be seen that the percentage recovery of soda lignin is influenced by the pulping condition⁶, the precipitation pH value¹¹ and the type mineral acid used. The yield of soda lignin precipitated from hydrochloric acid and nitric acid were in the range of 1.20-1.33 g for every 100 ml black liquor used, which is relatively low as compared to sulfuric acid and phosphoric acid (1.42-1.50 g/100 ml black liquor), indicating that yield is dependent on the number of hydrogen ions in the acid. The phosphoric acid, which has three hydrogen ions, gave the highest yield, follow by sulfuric, hydrochloric and nitric acid respectively, hence the preferable acid for precipitating soda lignin is phosphoric. It is also interesting to note that at low acid concentration (20% v/v), the yield of lignin consistently gave the highest value irrespective the type of acid used, which is probably due to the localized acidification or non-uniform precipitation effect when high acid concentration was used⁵.

Nitrobenzene oxidation is one of the standard procedures for analyzing lignin by chemical degradation technique in order to gain information about the composition of the original polymer. The composition of three monomeric lignin unit, viz p-hydroxyphenyl (H), guaiacyl (V) and syringyl (S) which are capable to produce the corresponding degradation product of p-hydroxybenzaldehyde, vanillin and syringaldehyde were analyzed on the results shown in Table 2.

Syringaldehyde was found to be the predominant followed by vanillin as a second major degradation product. The total yield of the oxidation products is ranged from 17.3% to 27.2%. The lower total yield of oxidation products for concentrated sulfuric acid and 60% nitric acid may be explained by a higher condensation degree of the lignin⁷ and may consist of some polysaccharide material such as xylose as shown in the C¹³ FT-NMR spectra. For soda EFB lignin, the ratio of S: V: H was 7-15:6-11:1 which falls in the same category as cereal straw or grass type lignin^{6,7}.

Typical IR spectra of soda lignin precipitation from various mineral acids are shown in Figure 1. The strong and broad band at 3433cm⁻¹ is the characteristic of OH group or phenolic compound. The band at 1330 cm⁻¹ is due to the bending of vibration in phenolic OH group, whereas the

Table 2: Yield and molar ratio of degradation products of the soda lignin by nitrobenzene oxidation

| | Sulfuric acid | | | Hydrochloric acid | | | Phosphoric acid | | | Nitric Acid | | |
|---|---------------|------|------|-------------------|------|------|-----------------|------|------|-------------|------|------|
| | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 |
| Acid Concentration (% v/v) | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 |
| Oxidation product (% w/w to the internal standard) | | | | | | | | | | | | |
| p-hydroxybenzadehyde (H ₁) | 1.1 | 0.8 | 0.6 | 1.0 | 0.7 | 0.8 | 1.1 | 0.9 | 0.7 | 1.0 | 0.9 | 0.8 |
| Vanillin (V ₁) | 9.6 | 8.6 | 6.2 | 9.9 | 7.9 | 9.0 | 10.2 | 9.3 | 8.3 | 7.9 | 5.5 | 5.7 |
| Syringaldehyde (S ₁) | 10.9 | 10.5 | 7.2 | 11.2 | 9.8 | 10.7 | 11.0 | 12.0 | 10.9 | 9.4 | 6.9 | 7.0 |
| p-hydroxybenzoic acid (H ₂) | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.3 |
| Vanillic acid (V ₂) | 1.0 | 1.0 | 0.5 | 0.8 | 0.9 | 1.1 | 0.8 | 0.9 | 1.2 | 1.0 | 1.0 | 1.1 |
| Syringic acid (S ₂) | 2.3 | 2.4 | 2.6 | 3.0 | 2.7 | 2.7 | 3.9 | 3.7 | 3.0 | 3.9 | 2.9 | 3.1 |
| p-coumaric acid (B) | T | T | T | T | T | T | T | T | T | T | T | T |
| Total | | | | | | | | | | | | |
| (H ₁ +V ₁ +S ₁ +H ₂ +V ₂ +S ₂ +B) | 25.1 | 23.6 | 17.3 | 26.2 | 22.3 | 24.6 | 27.2 | 27.0 | 24.3 | 23.5 | 17.4 | 18.0 |
| Molar ratio: S/H | 10.2 | 11.7 | 12.3 | 11.8 | 12.5 | 12.2 | 11.5 | 14.3 | 15.4 | 7.3 | 8.9 | 9.2 |
| V/H | 8.2 | 8.7 | 8.4 | 8.9 | 8.8 | 9.2 | 8.5 | 9.3 | 10.6 | 6.8 | 5.9 | 6.2 |
| H/H | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

T= Trace amount; S=S₁+S₂; V=V₁+V₂; H=H₁+H₂

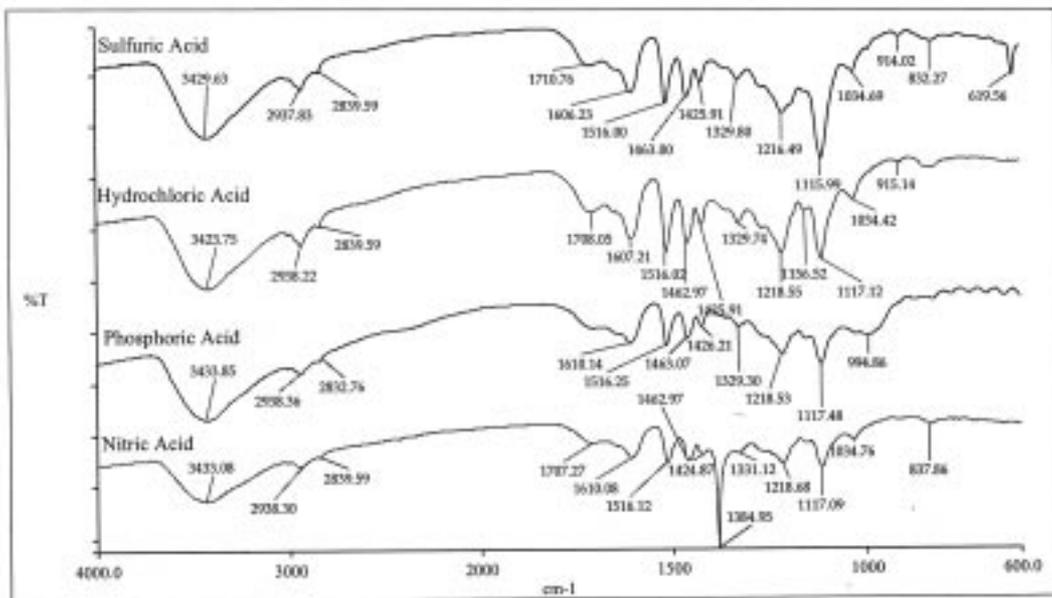


Figure 1 : Typical IR spectra of soda lignin precipitated from various mineral acids

band at 1030 cm^{-1} is characteristic of a primary alcohol¹⁰. The clear shoulder at $2940\text{-}2930\text{ cm}^{-1}$ and the band at 1464 cm^{-1} are assigned to CH stretching of methyl or methylene group. Broad medium band at 1710 cm^{-1} is due to the conjugated carbonyl stretching. Moreover, two bands at 1516 cm^{-1} and 1610 cm^{-1} are characteristic of aromatic rings due to the aromatic skeletal vibrations and the band at 1117 cm^{-1} is due to the ether stretching. A band at $830\text{-}840\text{ cm}^{-1}$ indicates the C-H deformation and ring vibration. The strong and sharp band at 620 cm^{-1} that is only present in the spectrum of lignin precipitated from sulfuric acid is due to C-S stretching, whereas the spectrum of lignin precipitated from nitric acid showed a strong and sharp band at 1385 cm^{-1} which is characteristic of an N=O stretching.

Bands at 1330 , 1220 and 1120 cm^{-1} are corresponding to syringyl unit, whereas small bands at 1275 , 1153 and 1037 cm^{-1} are assigned to guaiaxyl unit of lignin molecules¹². In general, the IR spectra of soda lignin isolated from different concentration levels were found to be the same suggesting that the general composition of lignin is not affected by the concentration and types of acids used.

The study of UV spectroscopy at $210\text{-}350\text{ nm}$ can be used to verify the purity of the precipitated lignin. From Figure 2, it can be seen that the maximum absorbance is at 235 nm , which is assigned to the phenolate ion compound. The spectra also showed that intensity of absorbance is related to the level of lignin concentration, the higher the absorbance value the purer is the lignin compound. A lower absorbance could be due to the co-precipitated of non-lignin material such as polysaccharide degradation product such as ash and salts⁶.

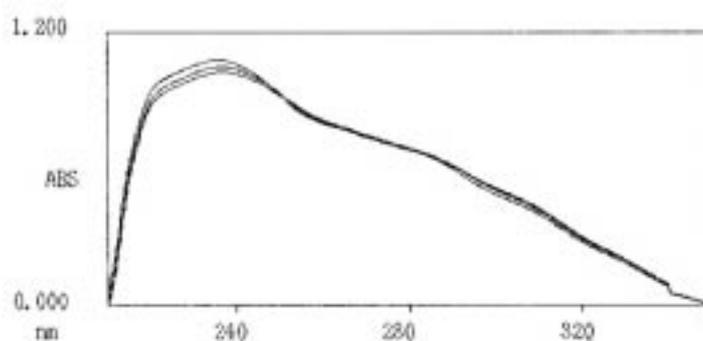


Figure 2 : Typical UV spectra of soda lignin precipitated from various concentrations of mineral acids

Table 3 : The absorbance and the maximum wavelength, $\lambda_{max}(nm)$ of lignin

| Concentration (v/v) | Absorbance / λ_{max} (nm) of lignin | | | |
|---------------------|---|-------------------|-----------------|-------------|
| | Sulfuric Acid | Hydrochloric Acid | Phosphoric Acid | Nitric Acid |
| 100% | 1.085/235.8 | 1.053/236.4 | 1.105/235.5 | 1.096/235.9 |
| 60% | 1.030/236.5 | 1.011/236.0 | 1.037/236.2 | 1.026/236.3 |
| 20% | 1.052/236.3 | 1.050/235.8 | 1.063/236.4 | 1.028/236.1 |

The absorbance values at the corresponding maximum wavelengths, I_{\max} were summarized in Table 3. It can be seen that the concentrated mineral acids regardless of their types gave the highest absorbance value followed by the 20% v/v and the 60% v/v. The phosphoric acid exhibited the highest absorbance value (1.105) followed by nitric acid (1.096), sulfuric acid (1.085) and hydrochloric acid (1.053) at I_{\max} of 235 nm.

From the GPC results, the weight-average (M_w), number-average molecular weights (M_n) and poly-dispersity (M_w/M_n) values are calculated as shown in Table 4. Lignin isolated by various mineral acids show no significant difference in their weight-average, which ranges from 2444 to 3279. This range is slightly higher compared to alkaline lignin extracted from wheat straw and ash-antraquinone lignin extracted from the EFB^{10,13}. The results also indicate that the weight-average molecular weights of lignin precipitated by concentrated mineral acid were consistently high due to the localized acidification and non-uniform precipitation effects⁵, with reasonably value of calculated M_w/M_n .

Table 4 : Weight-average and number-average molecular weights and poly-dispersity

| Concentration (%v/v) | Sulfuric acid | | | Hydrochloric acid | | | Phosphoric acid | | | Nitric Acid | | |
|----------------------|---------------|------|------|-------------------|------|------|-----------------|------|------|-------------|------|------|
| | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 | 20 | 60 | 100 |
| M_w | 2605 | 2930 | 3201 | 2872 | 2555 | 3274 | 2923 | 2444 | 3165 | 2974 | 3054 | 3279 |
| M_n | 2076 | 1731 | 1694 | 1895 | 2065 | 2018 | 1953 | 2163 | 2143 | 2279 | 2531 | 2580 |
| M_w/M_n | 1.23 | 1.69 | 1.89 | 1.52 | 1.24 | 1.62 | 1.60 | 1.13 | 1.48 | 1.31 | 1.21 | 1.27 |

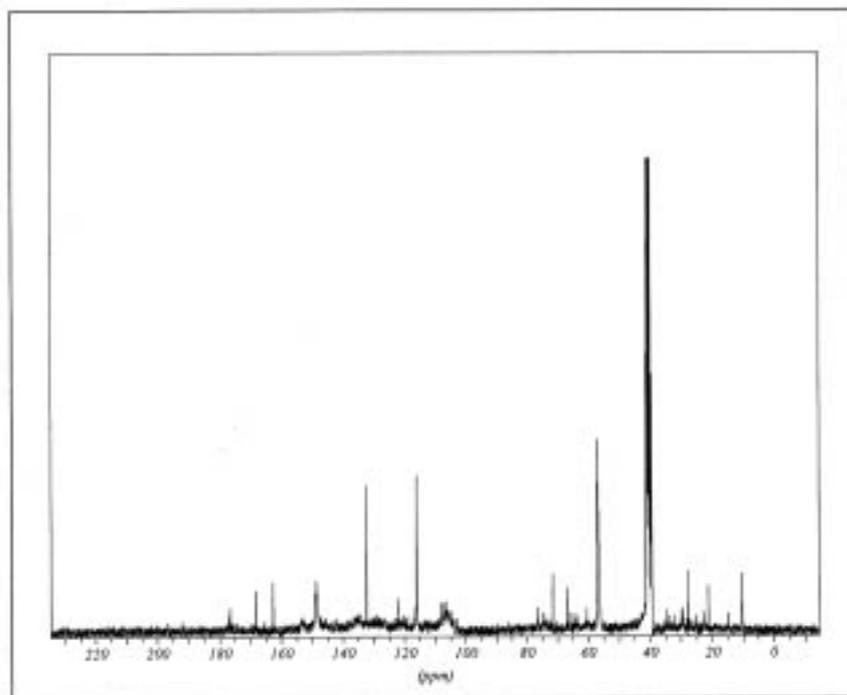


Figure 3 : C^{13} -NMR spectrum for lignin precipitated by 20% v/v sulfuric acid at pH_2

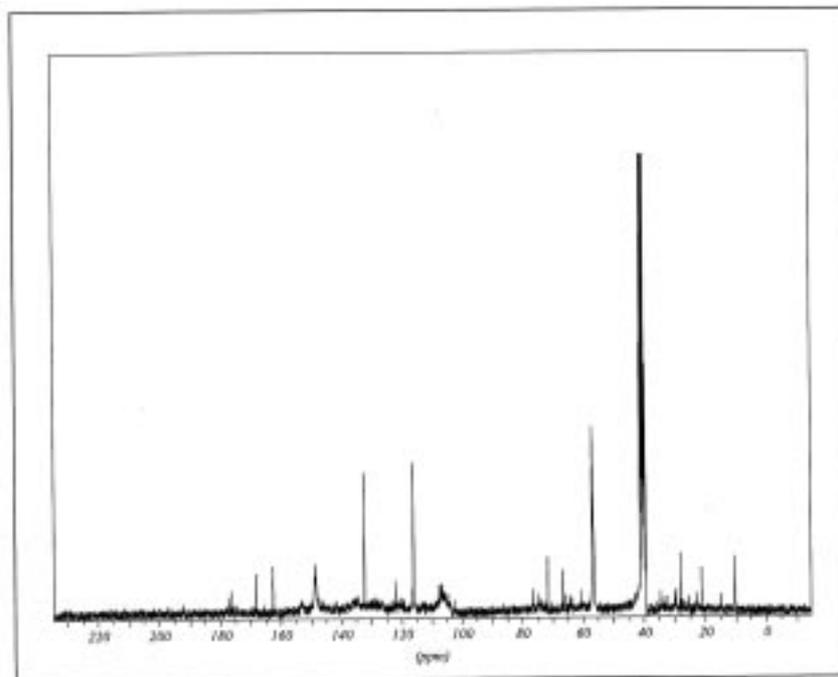


Figure 4 : ^{13}C -NMR spectrum for lignin precipitated by 20% v/v hydrochloric acid at pH_2

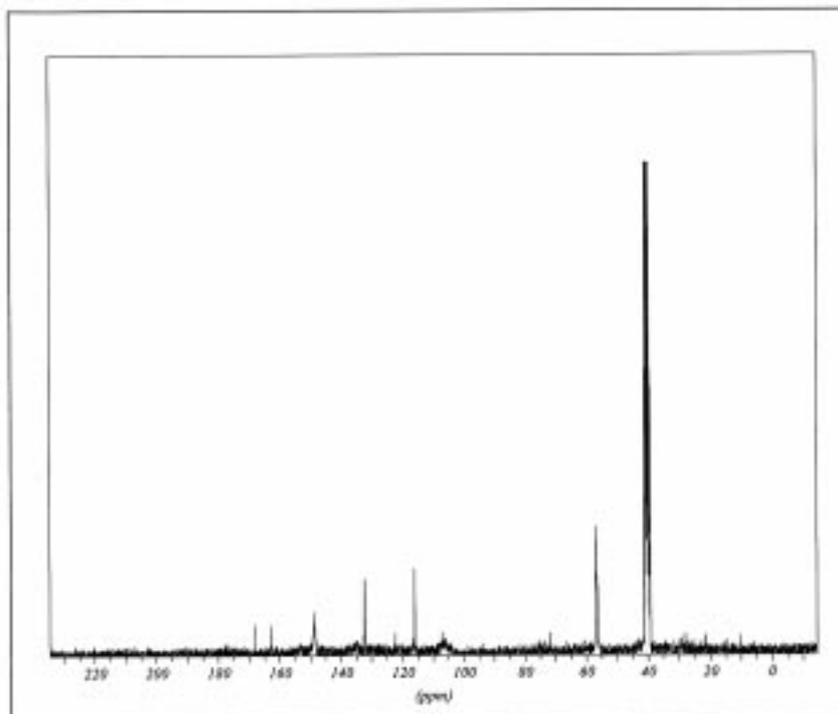


Figure 5 : ^{13}C -NMR spectrum for lignin precipitated by 20% v/v phosphoric acid at pH_2

Table 5: Carbon-13 chemical shift values (δ ppm) and signal assignment of soda lignin isolated by various mineral acids

| H_2SO_4 | | HCl | | H_3PO_4 | | HNO_3 | | Assignment |
|--------------|------|--------------|------|--------------|------|--------------|------|---|
| δ ppm | Int. | |
| | | | | | | 192.0 | vw | α -CHO in benzaldehyde |
| 168.1 | w | 168.1 | w | 168.3 | vw | 168.1 | w | CO in acetates of phenols |
| 162.5 | s | 162.6 | s | 162.6 | vw | 162.5 | s | C-4 in acetates of phenols |
| 153.2 | vw | 153.2 | vw | | | 153.2 | vw | C-3/C-3' in \bar{e} 5-5 unit |
| 149.0 | w | 149.0 | w | | | 149.0 | w | C-3,4 in $G\bar{e}$ |
| 148.7 | w | 148.7 | w | 148.8 | w | 148.7 | s | |
| | | 145.8 | vw | | | | | C-4 in $G\beta$ - <i>o</i> -4 $n\bar{e}$ |
| 135.0 | vw | 134.6 | vw | | | 135.0 | vw | C-1 in G,S with C4OR |
| 132.4 | vs | 132.3 | vs | 132.4 | s | 132.4 | vs | C-1 in $Gn\bar{e}$ |
| 130.1 | vw | | | | | 130.5 | w | C-1 in S with α -COOH |
| | | 129.3 | vw | | | 129.4 | vw | CH: olefinic and aromatic |
| 122.2 | w | 122.1 | w | 122.2 | vw | 122.2 | w | C-6 G -CH ₂ -; G-CH |
| | | | | | | | | olefinic carbons |
| | | 119.1 | vw | | | 119.1 | vw | C-6 G pinoresinol and |
| | | | | | | | | phenylcoumaran |
| 116.0 | vs | 116.0 | vs | 116.0 | vw | 116.0 | vs | C-5 in $G\bar{e}$ and $Gn\bar{e}$ |
| 108.0 | vw | 108.0 | vw | | | 108.0 | vw | C-2 vinyl ether |
| 107.1 | vw | 107.1 | vw | 107.1 | vw | 107.1 | W | C-2;C-6 in S with α -C=O |
| | | | | | | | | or α -C=C |
| 106.6 | vw | 106.6 | vw | | | 106.6 | w | |
| | | 104.5 | vw | | | 104.5 | vw | C-2;C-6 in S in general |
| | | | | | | 102.7 | vw | C-1 in xylose internal unit |
| | | | | | | 83.7 | vw | C- β in β - <i>o</i> -4 with α -CO |
| 76.2 | vw | 76.3 | vw | | | 76.4 | vw | |
| 75.5 | vw | | | | | | | C-4 xylose |
| 74.9 | vw | 74.9 | vw | | | 74.9 | vw | |
| | | 73.6 | vw | | | 73.5 | vw | C-3 xylose |
| 71.6 | s | 71.6 | s | 71.7 | vw | 71.6 | s | C- α in β - <i>o</i> -4 |
| | | | | | | 70.4 | vw | |
| 66.6 | w | 66.6 | w | | | 66.6 | w | C- γ |
| 65.7 | vw | 65.7 | vw | | | 65.7 | vw | |
| 65.5 | vw | 65.4 | vw | | | 65.5 | vw | |
| | | 63.8 | vw | | | 63.8 | vw | C- γ in β - <i>o</i> -4 |
| 60.4 | vw | 60.4 | vw | | | 60.4 | vw | C- γ |
| 56.9 | vs | 56.9 | vs | 56.9 | s | 56.8 | vs | O-CH ₃ in G and S unit. |
| 56.8 | vs | 56.8 | vs | | | | | |
| | | 56.5 | s | | | | 56.5 | s |
| 34.5 | vw | 34.6 | vw | | | 34.5 | w | -CH ₂ - (G-CH ₂ -CH ₂ -OH) |
| 33.6 | vw | 33.6 | vw | | | 33.6 | vw | -CH ₂ - |
| | | 32.1 | vw | | | 32.1 | vw | |
| | | 29.9 | vw | | | 29.9 | w | |
| 29.7 | vw | | | | | 29.7 | s | |
| | | | | | | 29.5 | vw | |
| 29.4 | vw | 29.4 | vw | 29.4 | vw | 29.4 | w | |
| 28.4 | vw | | | | | | | |
| 27.9 | s | 27.9 | s | 27.9 | vw | 27.9 | s | |
| | | | | | | 27.4 | vw | |
| | | | | | | 27.1 | vw | |
| | | 25.4 | vw | | | 25.3 | w | |
| | | 22.9 | vw | | | 22.9 | w | |
| 21.3 | W | 21.3 | w | 21.3 | vw | 21.3 | w | |
| | | 17.8 | vw | | | 14.8 | vw | -CH ₃ |
| 10.3 | s | 10.3 | s | 10.3 | vw | 10.4 | s | |

G=Guaicyl, S=Syngyl, \bar{e} =etherified, $n\bar{e}$ =non-etherified vs=vary strong, s=strong, w=week, vw=vary week

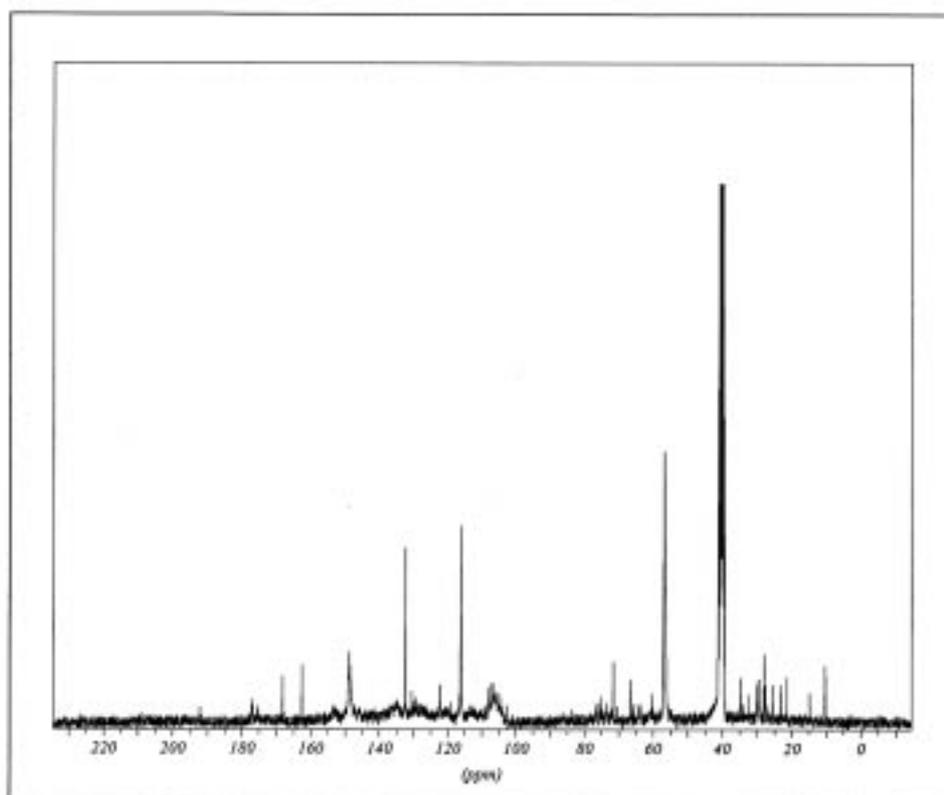


Figure 6 : C^{13} -NMR spectrum for lignin precipitated by 20% v/v nitric acid at pH_2

As evident from the spectra of C^{13} -NMR (Figures 3-6), there is no significant difference in the structure of lignin precipitated by various mineral acids, probably because of the same-based components present in the lignins. The assignment of the peaks as given in Table 5 were made according to the literature data for lignin dissolved in $DMSO-d_6$ ^{5,14-17}. The incomplete dissolution of the sample may cause the signal/noise to be significantly high as exhibited in the spectra.

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