Studies on the Poplar Deltoides Lignin Preparation and Effects on Its Structure Modifications

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ABSTRACT:

This paper examines the physico-chemical properties and structural features of thio lignin and alcohol lignin preparations extracted from fast-growing poplar wood. The lignin preparations were characterized using UV, IR and alkaline nitrobenzene oxidation methods. The yield was higher in thiolignin due to its preparation from wood under drastic alkaline conditions and almost the total amount of alkaline degraded lignin was precipitated except acid soluble lignin. In case of ethanol lignin, structural modifications were comparatively less and form a cream colored lignin more or less similar to its original natural color. The methoxyl values were higher due to syringyl unit present in hard wood lignin in addition to guaicyl unit present in soft wood. The higher values of methoxyl content of isolated lignin revealed that it was built up of high syringyl units. The elementary analysis, methoxyl group and hydroxyl groups were presented by C_9 formula indicated that it was made up of phenyl propane monomers. Nitrobenzene oxidation of thio lignin and ethanol lignin yield more or less the chromatograms of similar pattern, except difference in relative percentage. The ultra violet spectra of lignins were quite similar, irrespective of the source and method of isolation. Infrared spectroscopy studies of poplar deltoides, thio and ethanol lignin shown different absorption bands which have been utilized for structural investigations.

INTRODUCTION:

To study the characteristic and chemistry of lignin, it is desirable to isolate the lignin in high yields so that the chemical nature of lignin remains unchanged during isolation. The major portion of the lignin can be isolated by the chemical reactions which solubilized either the lignin or the polysaccharides. Lignin is an aromatic, amorphous material which forms a part of the cell wall and the middle lamella in wood. Lignin contains only carbon, hydrogen and oxygen. The constitutional model of lignin is composed of many reactive groups such as others of various types, primary and secondary alcoholic-hydroxyl groups, phenolic hydroxyl groups, carbonyl groups, methoxyl groups, ethylene linkages and aromatic sites of phenyl propanoid structures. Since the lignin macromolecule is susceptible to a wide variety of oxidants, the oxidation reactions have been arbitrarily classified in to three categories according to the degree of lignin degradation achieved. These comprises degrading lignin to aromatic carbonyl compounds and carboxylic acids degrading aromatic rings and limited to specific groups.

The chemistry of lignin is extremely complex. The lignin molecules are composed of building blocks having general structure of a six carbon ring with three carbons attached to one end, called phenyl propane or propyl benzene structure. (Figure -1) shows the basic units of lignin molecule. The combination of these building units can occur either through ether and or by carbon to carbon linkages.

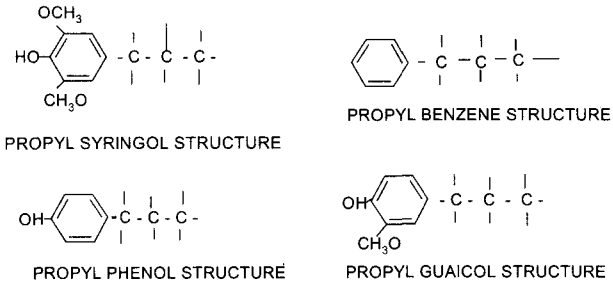

FIG. 1 Building units of lignin molecule.

Isolation of Ethanol lignin:

For the preparation of ethanol lignin from poplar, the method of Holmberg and Funius was followed with some modifications. 200 gm of extractive free poplar dust was placed in a five litre three necked round bottom flask, fitted with stirrer, nitrogen bubbler and reflux condenser and a dropping funnel. Ethanol (3L) containing HCl to a solution to acid concentration 0.2N was added to the flask slowly from the dropping funnel. The content of the flask were gradually heated to $85^{+}.2$ ^{0}C on a water bath for 2 hrs. A slow stream of N_2 gas at the rate of about 60 bubbles per minute was maintained throughout the experiment. The reaction mixture was cooled to 35^{0}C and was filtered on a buchner funnel. The dust was washed with pure ethyl alcohol until the washing was colorless. The filtrate and washings were combined and concentrated under reduced pressure at

35^0C. Lignin was precipitated by pouring the concentrated extract in a thin stream over a large excess of vigorously stirred distilled water and separated by centrifugation. The crude ethanol lignin was dissolved in pure dioxane and precipitating in to ether washed with fresh ether and twice with petroleum ether and finally dried in vacuum.

Thiolignin :

Thiolignin was isolated from kraft liquor which is a waste of majority of paper mills in the world. The main objective to study the kraft lignin or thio lignin was for its further modification in to industrial important compounds. Kraft digestion of poplar deltoids was carried out by cooking 200gms (O.D) chips with 20% chemicals at 170 ^{0}C in series digester consisting of six bombs each of 2.5lbs capacity. These bombs were cooled in cold water and black liquor was collected filtration through muslin cloth, suspended fibrous raw material from black liquor was removed by filtration through pulp pad. The black liquor was separated by filtration on a muslin cloth and acidified with dil.H_2SO_4 in two steps firstly up to PH-9 (while gently stirring to promote the release of hydrogen sulphide and carbon dioxide) and secondly to PH-3 after heating the suspension to 80^0 C. A heavy precipitate separated immediately which was filtered and washed successively with dil HCl and water since in an acidified water precipitate is colloidal. Finally, the precipitate was washed with distilled water, until free of chloride ions and freezes dried.

Purification of thiolignin :

The crude thiolignin was purified according to the method described by brauns. The crude thiolignin was dissolved in anhydrous dioxane and concentrated under reduced pressure. The dioxane solution was centrifuged to separate precipitated sulphure and carbohydrates and the clear solution was diluted with dioxane to give a 10% solution. The thio lignin was precipitated by adding the dioxane solution drop wise to vigorously stirred anhydrous ether. The precipitate was washed twice with fresh ether and then twice with pure benzene and finally twice with low boiling petroleum ether by centrifugation. The suspension of lignin in petroleum ether was kept overnight and centrifuged dried in vacuum dessicator over phosphorous pentaoxide. The thiolignin was exhaustively extracted in a soxhlet for 4 hrs with carbon disulphide, for the removal of any elemental sulphur. Finally repeatedly precipitated from dioxane in to ether until the methoxyl content remained constant.

Chemical characterization : Structural features of lignin :

A series of experiments were carried out in order to study the chemical constitution of lignin. It includes determination of carbon and hydrogen content, methoxyl content, hydroxyl content, carbonyl content and carboxyl content. The lignins were oxidized by alkaline nitrobenzene and the oxidation products were identified by gas chromatography. In addition to these studies infrared spectra of these lignins was also taken and molecular weight of lignins was determined.

a). *Carbon,Hydrogen and Oxygen :* Percentage of carbon and hydrogen were determined by hereus CHN rapid analyzer. The results are recorded in Table-1 .

b). *Methoxyl content :* TAPPI method T-209-SU-69 was applied for the determination of methoxyl content.

c*). Total hydroxyl content :* The total hydroxyl content (both aliphatic and aromatic) was conveniently determined by acetylation. The method involves two steps i). Acetylation of lignin and ii). estimation of acetyl content.

d). *Phenolic hydroxyl content :* Phenolic hydroxyl content was determined by ultraviolet spectroscopy method which was developed by Aulin Erdtman and has been simplified further by Goldschmid.

e). Carbonyl group : Carbonyl groups were determined by hydroxyl-amine-hydrochloride method of Geiger and Soderberg.

f). *Carboxylic group :* Carboxylic groups were determined by titrating a lignin solution in alcohol against standard sodium hydroxide using PH meter.

g). *Infrared spectra :* Infrared spectrum is one of the most characteristic properties of a compound. It provides a fingerprint for identification and a powerful tool for the study of the molecular structure.

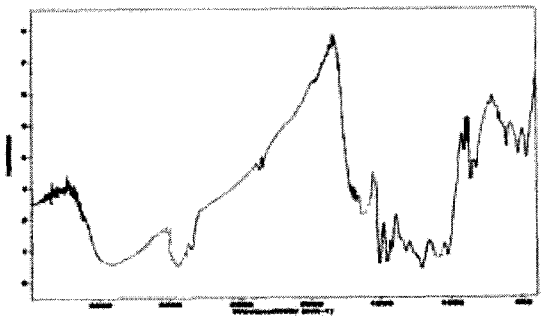

FIG. 2 IR spectra of ethanol lignin.

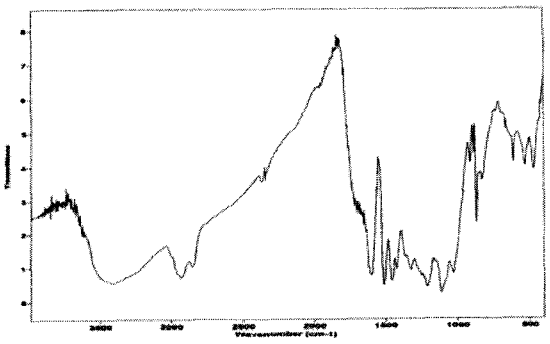

FIG. 3 IR spectra of thio lignin.

H). *Ultra violet spectra* : Lignin due to its aromatic nature, absorbs strongly in the ultra violet range of spectrum. In ultra violet absorption spectra of lignin and its derivatives, the absorptivity of the various structural elements are superimposed on each other. Although UV spectral studies are in general less informative regarding special structural features than those in the IR region, some intensity observations may be made when used as a means of comparing the isolated lignins and provide valuable tool in the identification of unsaturated organic compounds and in elucidation of structure.

Being phenolic in nature the lignin macromolecule is prone to oxidation by either homo or hetrolytic pathway depending on the oxidant and the reaction conditions used. (Waters, W.A. 1964) Oxidation of lignin to aromatic carbonyl compounds and carboxylic acids has served as a tool to structurally characterize lignin macromolecule in respect to its basic building units. The alkaline nitrobenzene oxidation transfer process of nitrobenzene degradation which is the quantitative method for determination of phenolic aldehyde which in turn relate to the basic building units of lignin macromolecule.

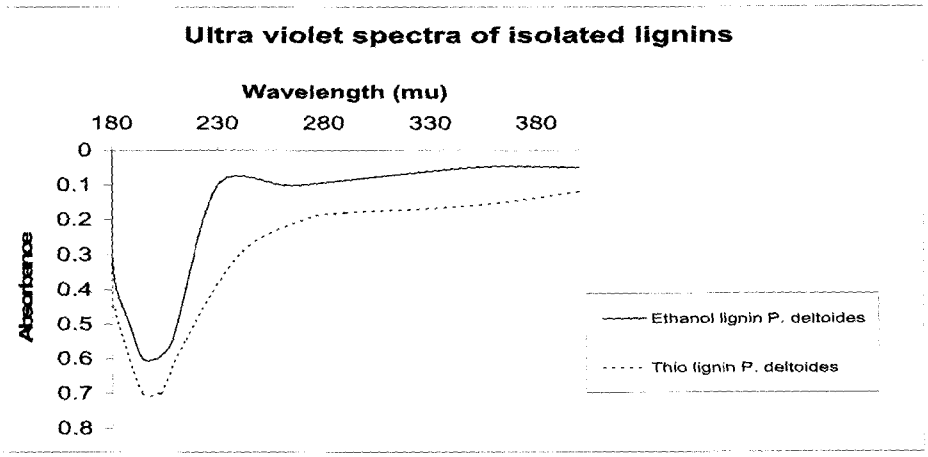

Fig. 4 UV spectra of thio lignin and ethanol lignin.

Experimental : The reaction was carried out in duplicate in stainless steel bomb of about 6 ml capacity taking about 100 mg lignin.The bombs are heated in a stationary digester half filled with water. Following conditions were maintained :

Lignin	100 mg
Reaction temperature ^{0} C	170^0 C
Nitrobenzene, ml	0.4
Sodium hydroxide (2N) ml	3

After heating for 3 hours at 170^0 C the bombs were taken out of digester. the contents of the bombs were transferred to a separating funnel. The nitrobenzene was removed by extracting the contents with pure ether till the ether layer becomes colorless. The reaction mixture was acidified with dilute HCl and extracted with ether two times. To the ether extract large amount of anhydrous sodium sulphate was added and kept overnight to remove traces of water. The sodium sulphate was then removed by filteration and the ether extracted filtrate was concentrated to dryness under vaccum.

Identification of oxidized products by GLC :

The oxidized product obtained were silylated as follows: Lignin sample (0.1 mg) was placed in a glass vial and 0.1 ml BSA (N<O-bis trimethyl silyl acetamide) and 0.2 ml pyridine were added to it. The vial was well capped and kept at room temperature for five minutes. Excess of pyridine was removed by passing dry nitrogen in to the mixture.Samples thus prepared were immediately chromatographed.

Analysis was performed on a perkin-elmer model 3920B. Chromatograph equipped with a glass column and flame ionization detector. The conditions were as follows:

Column	Glass column of 6`x 1/8`
Solid support	Gas chrome Q Aw (IDO-120 mesh)
Stationary phase	3% OV_{17}
Injector temperature (^{0}C)	230
Detector temperature	240

Column oven temperature^{0}C Linear temperature program	
Initial temp.(^{0}C):	150
Initial time, (min).	8
Rising rate (^{0}C/min)	8
Final temperature (^{0}C)	220

Final time, (min)	8
N_2 flow, (ml/min)	30
sensitivity	10 X
chart speed (cm/min)	0.5

Pressure for detector flame

Hydrogen (psi)	20
Air (psi)	50

Chromatograms are recorded in fig 5 and fig 6.

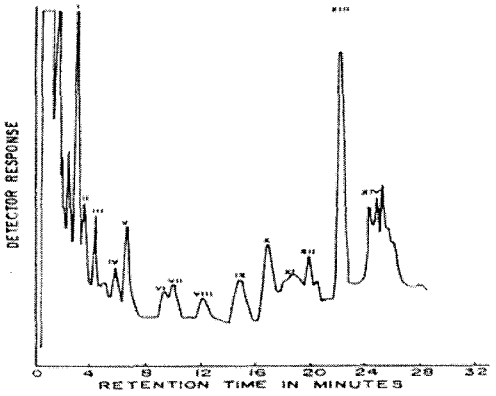

Fig. 5 GC of alkaline nitrobenzen oxidation

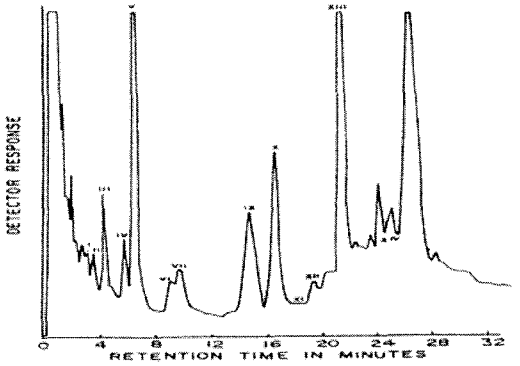

Fig. 6 GC of alkaline nitrobenzen oxidation products of thiolignin products of Ethanol lignin

Table –1 Composition of poplar deltoides lignins

S. No.	Type of Lignin	Carbon (%)	Hydrogen (%)	Oxygen (%)	Methoxyl (%)	Yield (%)	Klason Lignin (%)
1.	Thio Lignin	61.40	6.21	32.39	20.38	11.5	92.7
2.	Ethanol Lignin	58.82	6.48	34.70	21.80	5.9	89.3

Table –2 Functional groups of poplar deltoides lignins

S. No.	Type of lignin	Methoxyl (%)	Total Hydroxyl (%)	Phenolic hydroxyl (%)	Aliphatic hydroxyl (%)	Carbonyl per C_9
1.	Thio lignin	20.38	15.4	3.41	11.99	0.16
2.	Ethanol lignin	21.80	10.8	3.65	7.15	0.18

Table –3 Calculated C_9 formula of poplar deltoides lignins

S. No.	Type of Lignin	C9 Formula	C9 formula weight
1.	Thio lignin	$C_9 H_{9.0} O_{2.91} (OCH_3)_{1.06}$	196.46
2.	Ethanol lignin	$C_9 H_{9.37} O_{3.14} (OCH_3)_{1.5}$	214.11

RESULTS AND DISCUSSIONS :

Table –1 shows the yield and results of functional groups of isolated lignin from poplar deltoides. The yield of ethanol lignin was 5.9% while the yield of thio lignin was 11.5%. (Wu, S., 2003) The yield were higher in thio lignin was due to its preparation from wood under drastic alkaline conditions and almost the total amount of alkaline degraded lignin was precipitated except acid soluble lignin. (Wu, S., 2003)

The color of ethanol lignin was creamy while that of thio lignin was brownish black. The change in color was due to the drastic action of chemicals on lignin during alkaline cooking leading to generation of chromophoric groups in thio lignin. The main chromophores formed were: (i). –CH=CH- double bonds conjugated with the aromatic ring as depicted in fig-7. (ii) Quinon methides and quinons, which also serve as oxidative species creating chromophoric structure, (iii) Chalcones structures, (iv) free radicals. The two later structures are

likely to contribute to the color of thio lignin only to a minor extent. (Ziobro, G. C. 1990).

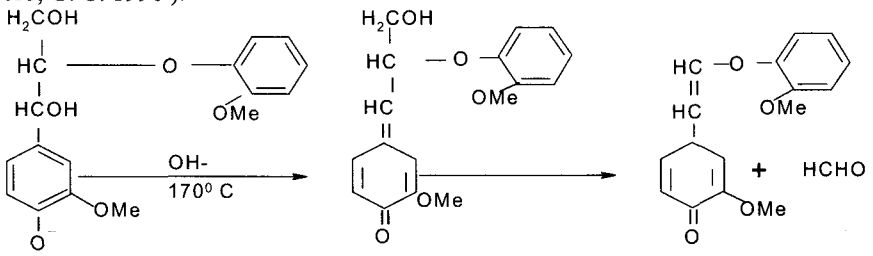

Fig. 7 Chromophoric groups in lignin

In case of Ethanol lignin, structural modifications were comparatively less and form a cream colored lignin more or less similar to its original natural color. The most characteristic functional group in lignin is its methoxyl group.. so it is used to trace the lignin in various connections. (Table –2) shows the methoxyl value of the isolated lignin of the poplar deltoides, 21.80 % for ethanol lignin and 20.38% for thiolignin. These methoxyl values were higher than that of soft wood and were comparable to other hard wood. The higher methoxyl content in hard wood lignin was due to syringyl unit present in hard wood of lignin in addition to guaicyl unit present in soft wood and hard wood lignins. The higher values of methoxyl content of isolated lignin shows that it was built up of high syringyl units. (Rozmarin, G.; 1976)

Another functional group in lignin was hydroxyl group. (Table-2) shows the total hydroxyl content of poplar deltoides for ethanol lignin is 10.8 % and for thiolignin is 15.4%. (Table-1) shows the elemental composition of isolated lignins. It shows that both lignins have higher carbon content, which indicate the aromatic nature of lignin molecule. Thio lignin is rich in carbon content than ethanol lignin while hydrogen and oxygen contents are comparatively low in thiolignin. Elemental and functional group analysis of thiolignin indicate that during kraft pulping lignin undergone severe structural modifications such as demethoxylation, chromophoric group formation, while the isolation of ethanol lignin is carried out at comparatively milder conditions and did not undergo drastic structural modifications. Therefore it could be concluded that ethanol lignin is certainly better lignin preparation for the structural studies. However the only disadvantage in the case of ethanol lignin was the yield. The results of elementary analysis, methoxyl group and hydroxyl groups were presented by C_9 formula as the lignin macromolecule was built up of phenyl- propane monomers.

Nitrobenzene oxidation of thio lignin and ethanol lignin yield more or less the chromatograms of similar pattern, except difference in relative percentage. The gas chromatograms of ethanol and thiolignin are given in fig-5 and fig-6. Analysis of both the chromatogram shows that it contains many unidentified peaks. Certain peaks may be assigned to the presence of partially and fully derived hydroxyl compounds arising from the degradation of residual carbohydrates but by considering other factors viz retention time, molecular weight and polarity of such compounds and investigation carried out by other workers on their model compounds, it seems highly improbable that all the unidentified peaks were derived from carbohydrate degradation products. Hence identification of other compounds in the chromatogram can be of immense help concerning lignin chemistry. (Bhandari, K.S.; 2002)

The comparision of relative percentage revealed that the yield of vanillin, P-hydroxyl benzaldehyde, syringaldehyde in thio lignin was considerably low in comparison to ethanol lignin. Particularly the yield of vanillin was drastically decreased from 14.48% to 4.23%. On the other hand, the yield of low molecular weight compounds were exceptionally higher in case of nitrobenzene oxidation of thiolignin. Low yield of vanillin, syringaldehyde and acetosyringene could be partially attributed to the formation of these molecular weight components. Disapearance of a major peak at RRT 4.11 for oxidation products of thiolignin also support the formation of relatively higher amount of low molecular weight compounds and reflects that due to structural modification during cooking, thiolignin may have become more prone to oxidation to produce low molecular weight compounds. It was observed that the yield of 5- carboxy vanillin was higher in thiolignin, which suggested that the fifth position of guaiacyl unit might have undergone condensation. The yield of carboxylic acids were either almost equal in both the cases or it improved in the case of thio lignin, as expected.

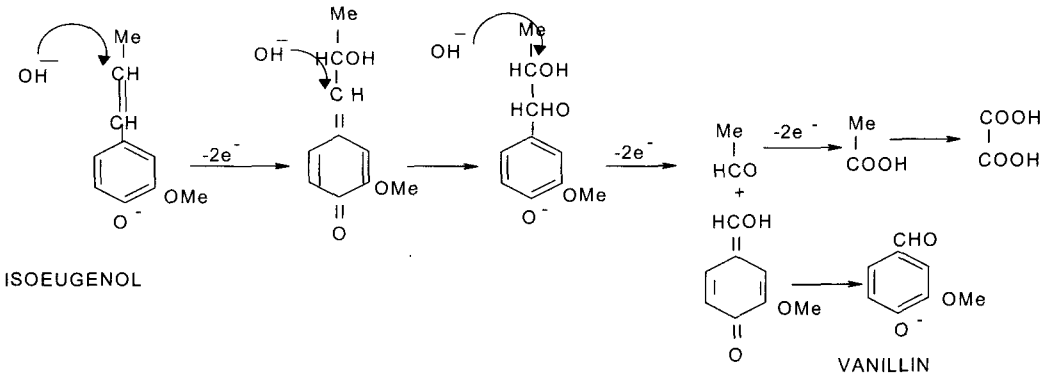

Fig. 8 Mechanism of Nitrobenzene Oxidation of Isoeugenol

The ultra violet spectra of lignin were quite similar, irrespective of the source and method of isolation. The typical lignin spectra decreases from a maximum near 205 nm with a pronounced shoulder near 230 nm. The minimum was followed by a characteristics lower maxima near 280 nm. However, the maxima and minima might have shift to higher or lower wavelength depending upon the nature of spectrum in which it had been recorded. The ultraviolet spectra of ethanol and thiolignin of poplar deltoides are shown in the fig-4. The maxima at 205 nm has been shifted to 215nm and the lower maxima also shifted to 245 nm and 300/280 nm. (Qian, P., 1992)

Infrared spectroscopy studies of poplar deltoides , thio and ethanol lignin (Fig-2 and Fig-3) shows different absorption bands which have been utilized for structural investigations. A wide and strong band at 3400 cm^{-1} is assigned to –OH stretching frequencies of strongly hydrogen bonded phenolic and alcoholic groups. The band at 1200 cm^{-1} represents the presence of phenolic hydroxyl groups. Absorption band at 2940 cm^{-1} were due to –OH stretching in methyl group. Shoulder at 2850 cm^{-1} was assigned to methoxyl group. The typical band at 1700 cm^{-1} representing carboxylic and β-carbonyl groups and at 1660 cm^{-1} representing α and β-acyl ketone which were very small in thio lignin indicating that a typical change had occured in the > C=O region of thiolignin. Two bands at 1600 cm^{-1} and 1500 cm^{-1} were characteristics of aromatic compound and were due to C=C vibrations of benzene ring. Band at 1456 cm^{-1} was due to bending C-H bonds. The bond at 1045 cm^{-1} indicates that the presence of dialkyl ether linkages. Thus the chemical analysis data revealing methoxyl, hydroxyl, syringyl and guaiacyl units were confirmed by the spectral analysis.(Sehgal, V.K., 1979)

CONCLUSIONS:

The lignin preparations were characterized using UV, IR and alkaline nitrobenzene oxidation methods. Infrared spectroscopy studies of poplar deltoides, thio and ethanol lignin shown different absorption bands which have been utilized for structural investigations. The ultra violet spectra of lignins were quite similar, irrespective of the source and method of isolation. The yield was higher in thiolignin due to its preparation from wood under drastic alkaline conditions and almost the total amount of alkaline degraded lignin was precipitated except acid soluble lignin. In case of ethanol lignin, structural modifications were comparatively less and form a cream colored lignin more or less similar to its original natural color. The methoxyl values were higher due to syringyl unit present in hard wood lignin in addition to guaicyl unit present in soft wood. The higher values of methoxyl content of isolated lignin revealed that it was built up of high syringyl units. The elementary analysis, methoxyl group and hydroxyl groups were presented by C_9 formula indicated that it was made up of phenyl propane monomers. Nitrobenzene oxidation of thio lignin and ethanol lignin yield more or less the chromatograms of similar pattern, except difference in relative percentage.

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