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Investigation of Lignins by FTIR Spectroscopy

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Summary: Applying special computer mathematical treatments to increase resolution of experimental spectra there were established a set of stable characteristic bands for isolated softwood lignins. In the 740–1620 cm^{-1} spectral range the band maximum positions did not change but values of bandwidths and peak intensities were varied in limits 15% and 32%. After analysis of the infrared spectra of 30 investigated samples a softwood lignin spectral model was constituted. This model allowed to clear discrepancies in the bands parameters of different mild isolated (lignins of Bjorkman, Pepper and Freudenberg), dioxane and technical lignin spectra. It was helpful for studying lignin structure changes during degradation procedures.

Keywords: degradation; FTIR spectroscopy; mild isolated and technical lignins; softwood lignin; spectral model; structure

Introduction

Lignins are polymeric aromatic constituents in plant cell walls. They are traditionally considered to be dehydrogenative polymers from three monolignols: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (see Figure 1). After structure lignins have been grouped into several types, characteristic of hardwoods, softwoods and grasses. The hardwood (angiosperm) lignins are built from the guaiacyl propane units (the abbreviation is G) and the syringyl propane units (the abbreviation is S). Lignins obtained from straws are built from the guaiacyl propane units, the syringyl propane units and 4-hydroxylphenyl propane units (the abbreviation is H).[1,2] The softwood (gymnosperm) lignins are built from the guaiacyl propane units (92–95%) with small amount of the syringyl propane and 4-hydroxylphenyl propane units (3–8%).[3] These aromatic building units are linked with a variety of ether and carbon-carbon bonds. The predominant linkage is the so-called β-O-4 llinkage. About 40–60% of all interunit linkages in lignin is via this ether bond. Different types of linkages between phenyl propane units form three-dimensional net structure and makes it difficult to completely degrade non-regular lignin macromolecules.

Because of structural complexity there are not uniformic knowledges about chemical and physical structure of this polymer and it is possible to consider only chemical lignin models. These models are based on analysis of degradation products from softwood and hardwood lignins. Such structural models with different amount of phenyl propane units having good agreement with the various analytical data in the lignin chemistry were proposed.[4–6] The fragment of one such softwood lignin model[6] is presented in Figure 2.

Recent investigations[7–8] pointed out on existence of some organization of lignin macromolecules in cell wall. Raman microprobe studies of secondary walls in black spruce revealed that phenyl rings of lignin are aligned preferentially in the plane of the cell walls[7] and this observation was supported by dynamic FTIR spectroscopy investigations.[8]

Considerable part of articles is devoted to studying so named technical lignins

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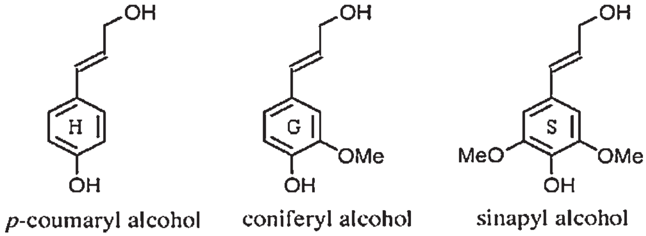

Figure 1.
The monolignols.[1]

that are ones extracted in severe or industrial conditions during delignification of wood.

At studying native (in situ) lignins two main problems occur. The one is searching isolation methods which do not change strongly structure of lignin. Lignins isolated under mild conditions are believed to be close to structure of native lignin after chemical data.[1]

The second problem is connected with selecting experimental method of analysis. A number of chemical and physical methods used for lignin structure characterization are destructive.

We assume that FTIR spectroscopy is non-destructive and most informative method of lignin investigation. The method opens perspective to find structural discrepancies in lignins isolated by different methods. Molecular spectra see differences in chemical structure and physical organization of different lignins that it is invisible for other analytical methods.

There are many articles devoting to FTIR spectra of lignins but full band interpretations are under the discussion.

In the work we used a new computer program method for enhancing lignin spectral information. It allowed revealing a complete lignin spectral band set in the diapason 740–1840 cm^{-1} and find some dependencies in behavior of spectral and structural parameters.

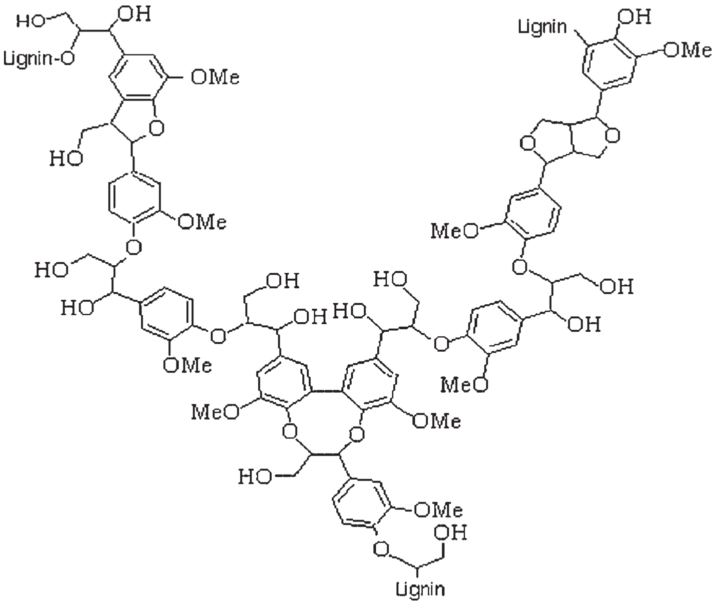

Figure 2.
Fragment of the softwood lignin model.[6]

Experimental Part

In the present work some softwoods mild isolated lignins and technical (kraft) lignins were investigated. There were three samples of mild extracted lignins (lignins of Bjorkman, Pepper and Freudenberg), dioxane lignin and 20 laboratory cook lignins extracted under different conditions. Three mild extracted wood lignins and dioxane lignin were extracted from spruce by standard methods.[1]

Technical lignins were isolated from black liquors of laboratory kraft cooks of spruce and fir woods.[9]

Lignin Isolation and Characterization

Bjorkman's lignin or milled wood lignin (MWL) was prepared in accordance with the method of Bjorkman.[10]

Milled wood was stirred for two hours with dioxane-water (9:1 by volume) that dissolved crude milled wood lignin. After removal of solvent from the filtrate, the crude preparation was purified by precipitation of a solution in 90% acetic acid into water. The product was further purified by dissolving into 1,2 dichloroethane-ethanol mixture (2:1 by volume) and precipitation into ethyl ether. The purified milled wood lignin required extensive drying under vacuum to bring it to a water-free condition.[1]

Pepper's lignin and dioxane lignin used in the present work were extracted from spruce wood by means of Peper's method.[11]

Peper's lignin was isolated by treatment of wood at elevated temperatures by a 9:1 mixture of dioxane and dilute hydrochloric acid (about 1 hour) in Argon atmosphere.

Dioxane lignin was isolated by treatment of wood at elevated temperatures by a 9:1 mixture of dioxane and dilute hydrochloric acid (more than 1 hour) in air atmosphere.

Freudenberg's lignin or cuoxam lignin was isolated by alternating treatments with boiling 1% sulfuric acid (1–2 h.) and extractions with copperammonia (12 h.). Four to five hydrolysis-extraction treatments were necessary to obtain reasonably carbohydrate-free preparations.[1]

Technical Lignins

Technical lignins were isolated from black liquors of kraft cooks of spruce and fir chips of different duration (cook duration was: 45, 75, 105, 120, 135, 150, 165, 180 minutes). The active alkali charge for the kraft cook was 17% (as Na_2O), the sulfidity was 25%.

All laboratory cooks of spruce and fir chips were carried out in a series of rotating autoclaves. Every 30 min from the beginning of the cook at 80 °C (every 15 minutes at 170 °C) a autoclave was withdrawn from the glycerin bath and was quickly cooled. The temperature was increased from 80 °C to 170 °C for 120 min., and the temperature was held at 170 °C for 60 min.

Dissolved lignins were isolated by a process of acidification of the black liquors with diluted sulfuric acid to pH 2.0, followed by the samples being centrifuged, washed repeatedly with water and dried by P_2O_5 in vacuum.

Reduction with Sodium Borohydride

Procedure of lignin reduction with sodium borohydride $NaBH_4$ was described in.[12]

The Functional Groups

The determination of content of phenolic hydroxyl groups in the investigated samples was performed by aminolysis according to.[27,28]

Content of the inter-unit alkyl – aryl ether bonds (α-O-4 and β-O-4 linkages) was calculated by the special developed method.[13]

The FTIR Spectroscopy

The FTIR absorption data were obtained by using a Bruker IFS-113v Fourier Spectrometer in spectral range 740–1840 cm^{-1} (resolution 2 cm^{-1}, number of scans 150). The lignin powder samples of 1–2 mg in weight were pressed with 300 mg of KBr powder as disks. For all spectra base line was constructed as linear line going through two points with minimal absorption around wavenumbers 740 and 1840 cm^{-1}. Then such corrected spectroscopic data were normalized on integral absorption over the considered spectral range.

Fitting Procedure

After our practice the most suitable mathematical model used for the non-linear least-squares fitting of the FTIR lignin spectra includes superposition of two line types, Lorentzian and Gaussian (equation (1)). The two functions corresponding to the same value of k (band number) are given the same wavenumber and the same full width at half height. A simulated spectrum $F(\nu)$ for N observed bands is as following[14]:

$$F(\nu) = \sum_{k=1}^{k=N} I_k \left(p_k e^{-\left(4\ln 2\frac{(\nu-\nu_k)^2}{\sigma_k^2}\right)} + (1-p_k)\frac{\sigma_k^2}{\sigma_k^2+4(\nu-\nu_k)^2} \right) \tag{1}$$

In this model, each band is characterized by four parameters: the wavenumber (ν_k), the bandwidth (σ_k) or full width at half height, the symmetric band intensity (I_k) and weights of Gaussian (p_k) and Lorentzian ($1-p_k$).

Fitting procedure consists in optimization of the band parameters in the function $F(\nu)$ to have minimal value of the expression (2):

$$\sum_{i=1}^{N1} (S(\nu_i) - F(\nu_i))^2 \tag{2}$$

where $S(\nu_i)$ is an experimental spectrum curve and N1 is a number of points in this spectrum.

The computer program work is stopped when for all points of the treated spectral range the root-mean-square deviation of simulated spectral curve from the experimental one was less than 1/200 of the mean intensity value. Thus there is obtained the high quality simulated spectrum with the determined band parameter sets.

Before using this fitting procedure an establishing a number of elemental bands was produced. Fixing wavenumbers for established number of elemental bands was made after studying Fourier self-deconvoluted[15–16] spectra of studied lignins and spectra of second derivative. It was made by detail analysis and comparison of origin softwood lignin spectra with Fourier self-deconvoluted and second derivative spectra. As a result the set of elemental bands in spectra of softwood lignins of different isolation in spectral range 740–1840 cm^{-1} was established.

A peculiarity of this program was using second derivative of experimental spectral curve as for fixing band wavenumbers (ν_k), peak intensities (I_k), bandwidths (σ_k) and G/L contribution coefficients (p_k) so and at optimization process.

Results

In the earlier investigations of softwood lignin spectra the row of absorption bands was found: 1720, 1670, 1590, 1500, 1463, 1423, 1360, 1330, 1270, 1215, 1140, 1125, 1087, 1031, 970, 855 and 815 cm^{-1}.[1,17,19,20,29] Using mathematical treatment of lignin spectra together with these bands we revealed some new peaks.[18]

Results of spectroscopic investigations of different lignin samples allowed to establish a set of stable characteristic bands. This set consists of 26 elemental symmetric bands in the 740–1620 cm^{-1} spectral range and 2–4 bands in the 1620–1800 cm^{-1} one. The parameters of elementary bands of the set were optimized by specially created computer procedure for the best decomposition of experimental lignin FTIR spectra on their constituent bands. The computer program was stopped when simulated spectral curve was close to the experimental one in all considered spectral range. Thus for each studied lignin we have got the high quality simulated spectrum with the definite set of elementary bands. For example, the FTIR absorption spectrum of Bjorkman's lignin and its band model are presented in Figure 3.

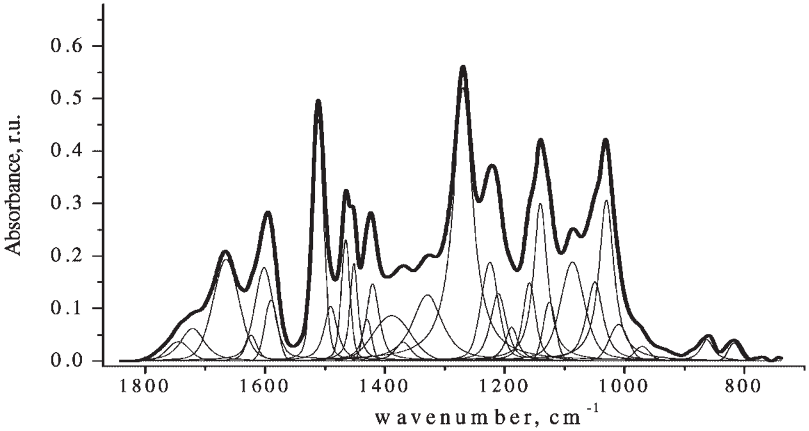

Figure 3.
The original spectral curve of Bjorkman's lignin (thick line) and its model components (thin line).

From physical point of view the elementary bands can be attributed to determined type of molecular vibrations of the lignin molecule units and existing interpretation of some bands can be found in literature.[1,17,19,20]

Analysis of the band parameters of the model after its applying to describe investigated lignins spectra showed that the band maximum positions in the 740–1620 cm^{-1} range did not change (the shift limits are ± 2 cm^{-1}), but values of bandwidths and peak intensities were varied in limits 15% and 32%.

This mathematical model is sensitive and it allows to assess small changes in the structure of softwood lignins which we observe by the molecular spectrum. To demonstrate high sensitivity of this approach an applying this spectral model to describe influence of reducing on lignin structure is presented.

So, for example, at studying process of reduction of Pepper's lignin sample small changes in its spectrum after reducing with $NaBH_4$ can be observed in Figure 4. The model band parameters showed that no significant changes occurred during this treatment. Reducing resulted to transformation of C=O groups to COH ones and it gave decreasing absorption bands at 1664 and 1720 cm^{-1}. Intensity of the model band at 1720 cm^{-1} dropped more than two times. Sarkanen *et al.*[21] assigned the first peak to νC=O of conjugated groups and that at 1720 cm^{-1} was assigned to nonconjugated moieties. It is worth to note that there was observed more than two times increasing intensity of the elemental model band near 1010 cm^{-1}. From this observation we propose that this band can be assigned partly to aliphatic hydroxyl groups vibrations.

Detail analysis of molecular spectra of three mild isolated lignins (lignins of Bjorkman, Pepper and Freudenberg) using their model band parameters allowed to reveal following spectral differences. For example, isolation procedure for Freudenberg's lignin is so that elementary bands of Freudenberg's lignin are broader and it results to decreasing intensities of the strong bands near 1510, 1465, 1270, 1140, 1030 cm^{-1}. These bands are the most intense ones in the molecular spectra of the G-type (guaiacyl kind) lignins. Peculiarity of Freudenberg's lignin spectrum is that the band at 1010 cm^{-1} is more than two times higher in comparison to two other mild isolated softwood lignins. Lignin of Bjorkman spectrally is more close to Pepper's lignin but some discrepancies are observed. It is possible that the enhanced band near 1660 cm^{-1} showing higher content of C=O groups conjugated with aromatic rings leads to some redistribution

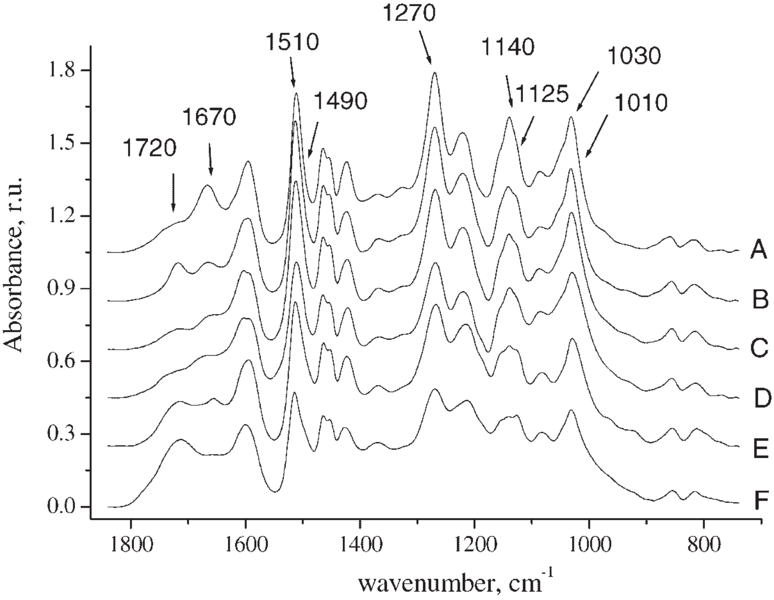

Figure 4.
FTIR spectra of extracted softwood lignins in the diapason 740–1840 cm^{-1}. From top to bottom: Bjorkman's lignin (A); Pepper's lignin (B), reduced Pepper's lignin (C), lignin of Freudenberg (D), dissolved Freudenberg's lignin (E) under the kraft cooking treatment, technical lignin dissolved after 150 minute kraft cooking (F).

in strong bands intensities - decreasing intensity of the bands near 1510, 1450, 1210 cm^{-1} and increasing intensities of the bands at 1590 and 1140 cm^{-1}.

So, there were revealed spectral differences in molecular spectra of three mild isolated lignins showing influence isolation method on lignin structure.

Studying influence of alkali cook conditions allowed to obtain new information about spectral manifestation of destruction processes of lignin.

Influence of degradation process on polymer structure was studied by comparing molecular spectra of mild isolated lignins and technical ones. Special laboratory treatment of Freudenberg's lignin close to traditional kraft cooking was done. Figure 4 shows the FTIR spectra of Freudenberg's lignin (curve D) and dissolved Freudenberg's lignin under the kraft cooking treatment (curve E).

Although many changes are seen not enough clearly in the spectra, the parameters of spectral model bands showed essential changing structure during degradation. These data showed increasing intensities of the bands at 1664 and 1713 cm^{-1}. These bands arise due to destruction process of lignin macromolecules and were attributed to vibration of C=O bonds in carbonyl and carboxyl groups.[1,21]

This alkali treatment gives more than two times increasing intensity of the band near 1490 cm^{-1}, some increasing bands at 1450, 1210, 1188 cm^{-1} and significant dropping the bands at 1140, 1050 and 1030 cm^{-1}.

It is well known that the β-O-4 type of structure (structure (1) in Figure 5) constitutes the major type of structural element in lignins. About 40–60% of all interunit linkages in lignin is via this ether bond.

As it was established[13,22–24] that base reaction at alkali treatment consists in cleavage of aryl-ether bonds what brings about an increase of phenolic hydroxyl content. These reactions lead to destruction of lignin macromolecules. A proposed mechanism for the alkali treatment is shown in Figure 5. After this reaction of degradation of lignin

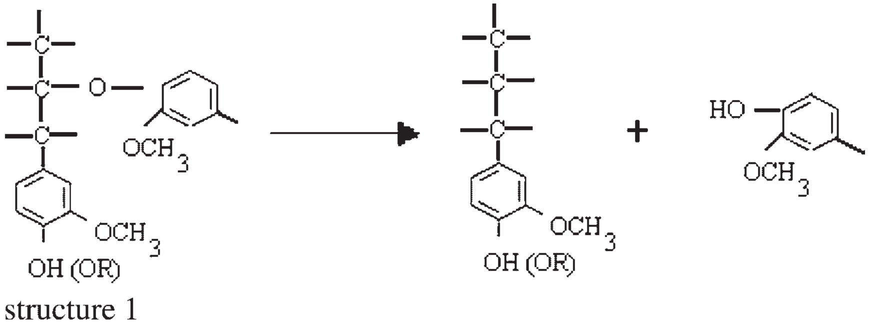

Figure 5.
A proposed mechanism for the alkali pulping. The major types of structural element in lignins – structure 1.

macromolecules the possible process is process of forming carbonyl and carboxyl groups. For example, appearing coniferaldehydes after cleavage of alkyl - aryl ether bonds was observed.[1]

Chemical analysis of the Freudenberg's lignin[13,24] showed that it has 52 aryl-ether bonds and 18 phenolic hydroxyl groups per 100 phenyl propane units (per C9-units). The dissolved during kraft treatment Freudenberg's lignin contains 22 aryl-ether bonds and 52 phenolic hydroxyl groups per C9-units. So we see that treated Freudenberg's lignin has approximately in 2.8 times more content of phenolic hydroxyl groups and approximately in 2.4 times less aryl-ether bonds.

From these chemical data changes we can possibly conclude about assigning the band near 1490 cm^{-1} to phenolic ring vibration and the bands at 1450, 1210, 1188 cm^{-1} to phenolic ring or phenolic hydroxyl groups vibrations. Decreasing aryl-ether bonds can result in dropping the bands at 1140, 1050 and 1030 cm^{-1}.

Analysis of spectra of dissolved lignins at various stages of cooking showed the same changing in theirs spectra as in spectrum of dissolved Freudenberg's lignin under the kraft cooking treatment.

It is worth to notice that elemental bands of technical dissolved lignins are broader and comparatively lower absorption at 1510, 1465, 1270, 1140, 1030 cm^{-1} was observed. In comparison with band parameters of mild isolated lignins there was revealed that degradation of lignin structure resulted to increasing bands connected with carbonyl and carboxyl groups and bands near 1490, 1450, 1210, 1188 cm^{-1} and lowering intensity of 1140, 1050 and 1030 cm^{-1} bands. There was revealed that similar directed changes of band parameters in the spectra of dissolved technical lignins depended on temperature and cook time.

It can be suggested that the spectral variations expose the changes in relative content of phenol-hydroxyl and aryl-ether bonds in lignins extracted at different stages of cooking. These results are well confirmed by investigation of structure of dissolved lignin by chemical methods which showed that cleavage of aryl-ether bonds brings about an increase of phenolic hydroxyl content.[13,22–24]

Chemical analysis of native lignin sample (lignin in wood) showed that it has 66 aryl-ether bonds and 13 phenolic hydroxyl groups per 100 phenyl-propane units but the dissolved technical lignin[13,24] contained 12 aryl-ether bonds and 66 phenolic hydroxyl groups per 100 C9-units.

It is interesting that the relation of intensities of two bands originating from the same vibration mode for two different aromatic ring types at 1490 and 1510 cm^{-1} changes more than two times for the presented samples. The value of this relation is connected with the transformation of one type of non-phenol aromatic ring to phenol type during the investigated treatments and can be used to fast evaluation of phenol hydroxyl groups. Relation of intensities of two bands at 1140 and 1125 cm^{-1} changes more than two times. The band near 1140 cm^{-1} perhaps can be attributed to vibration of aryl-ether bonds but the second band at 1125 cm^{-1} may be assigned to vibration bonds in forming condensed structure.

Conclusions

By computer treatment of the spectral data a mathematical model of softwood lignins was established. Its using for many of softwood lignin samples investigation showed a good reproducability and accuracy.

The model set of bands allowed to reveal discrepancies in spectral parameters of three mild isolated softwood lignins (lignins of Bjorkman, Pepper and Freudenberg) showing influence of isolation procedure on their structures.

The band parameters cleared changes during degradation treatment. It was shown that increasing and decreasing intensities of some bands go directly with increasing treatment time and temperature.

Significant discrepancies in the content and state of carbonyl and carboxyl groups were found in spectral region 1660–1720 cm^{-1}. This spectral range allowed evaluating degree of degradation of each studied lignin sample.

This method allows to systematizate lignins after theirs structure.

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