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MULTIWAVE UV/VIS DETECTION AT SEC ANALYSIS

Evaluation of Reactive Sites and Polyconjugated Substructures over MW Distribution of Lignins

A method for the assessment of the chemical heterogeneity of lignin has been proposed. It is based on the calculation of chemical composition distribution curves from the data obtained using the multiwave UV/Vis detection during SEC analysis. Feasibility of the method developed was demonstrated by investigation of a set of sulphur free soda lignins. The significant difference both in content and localization of conjugated phenolic and heteroaromatic structures attributed to MWD was found for lignins under study.

Chemical heterogeneity of isolated lignins including that attributed to molecular weight distribution (MWD) is now clearly recognized. At the same time, the data of chemical and most physico-chemical analyses of lignin being integral characteristics often cannot reveal this important lignin feature. Dual-wave UV detection has been proposed for lignin characterization by its degree of oxidation and for monitoring the distribution of colored structures over the MWD of pulp [1-3]. At the Latvian State Institute of Wood

Chemistry a novel approach has been developed based on the multi-wave UV detection at SEC analysis and calculation of chemical composition distribution (CCD) curves [4-7]. The CCD curves show the concentration profile of functional groups and substructures (quinones, stilbenes) as well as charge transfer complexes (CTC) over the molecular mass distribution (MMD) of the sample and allow to elucidate the details of isolated lignins heterogeneity in the aspects non-available at present for other methods of investigation.

In the present work a set of non-wood plants (bagasse, flax, straw, hemp, sisal, jute and abaca) soda lignins were characterized in terms of their chemical heterogeneity on the basis of CCD curves families.

Experimental

1) Commercially available soda lignins isolated by sulphur-free sodium hydroxide pulping from agricultural plants (flax, wheat straw, abaca, jute, sisal, hemp and bagasse) and purified by Granit's LPS (Lignin Precipitation System) were used as the object of the present investigation.

2) SEC analyses were carried out using chromatograph (Gilson, USA) with an UV/Vis- and RI-detectors connected in line. Chemical heterogeneity was studied using Jourdi

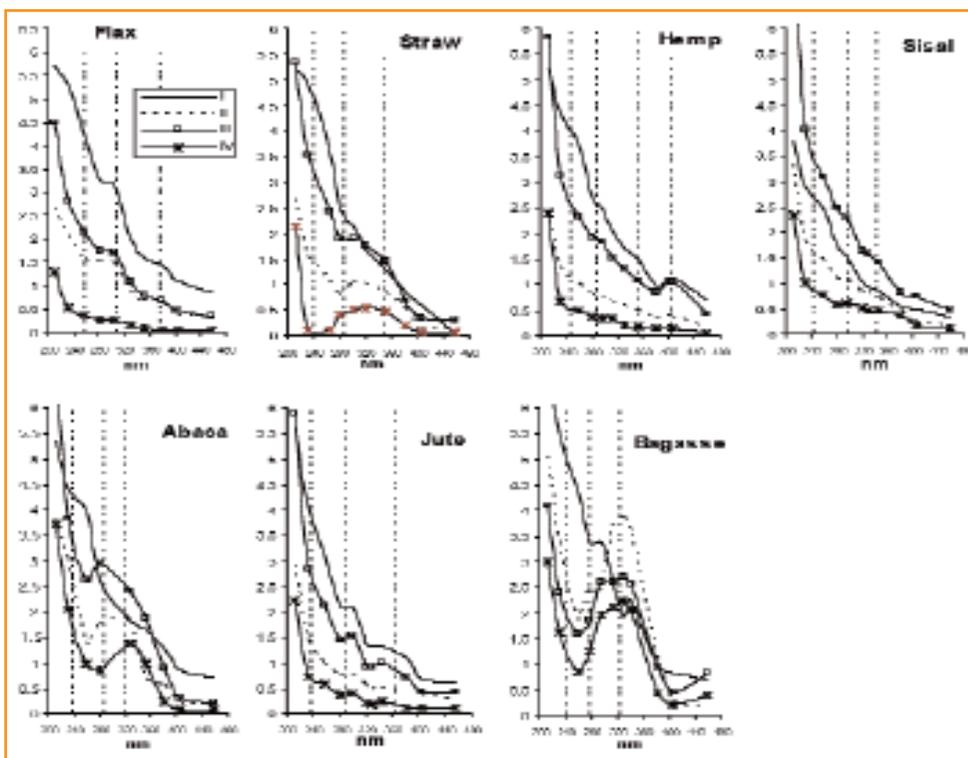


Fig. 2 - UV/Vis spectra calculated for MMD peaks I-IV as UV/RI from SEC chromatograms

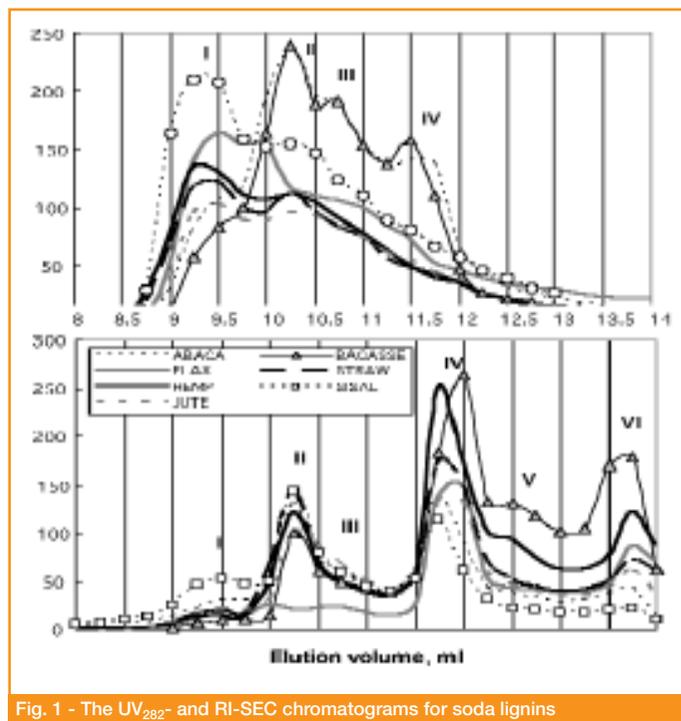


Fig. 1 - The UV_{282} and RI-SEC chromatograms for soda lignins

GEL GBR Mixed bed linear column (Alltech, USA) and 50 mM NaOH as an eluent. Analyses were carried out at ambient temperature. Injection volume 50 μ l of 0.1% lignin solution in 10 mM NaOH. Multi-wave UV-detector was operated at 212, 235, 260, 282, 296, 320, 328, 365, 380, 405 and 457 nm (2×10^{-5} AUFS). The wave-lengths for SEC monitoring were chosen based on the first derivatives of lignin samples UV/Vis spectra obtained using a Spectra 2000 (JENA, Carl Zeiss, Germany). The chemical composition distribution (CCD) curves were evaluated as described previously [6].

3) An ionisation difference UV-spectroscopy was used in order to estimate proportions of α -conjugated and weakly acidic groups in addition to the total content of phenolic hydroxyl groups [8].
4) The lignin π -conjugation systems were characterized using the value of mean conjugation length, which was estimated as number of CH-fragments from the ESR spectra line width. ESR spectra were recorded on an ES 1006 X-band recorder using the instrumental parameters described elsewhere [9].

5) The lignin antioxidant activity was characterized in terms of radical scavenging capacity (RSC) using a test with DPPH free

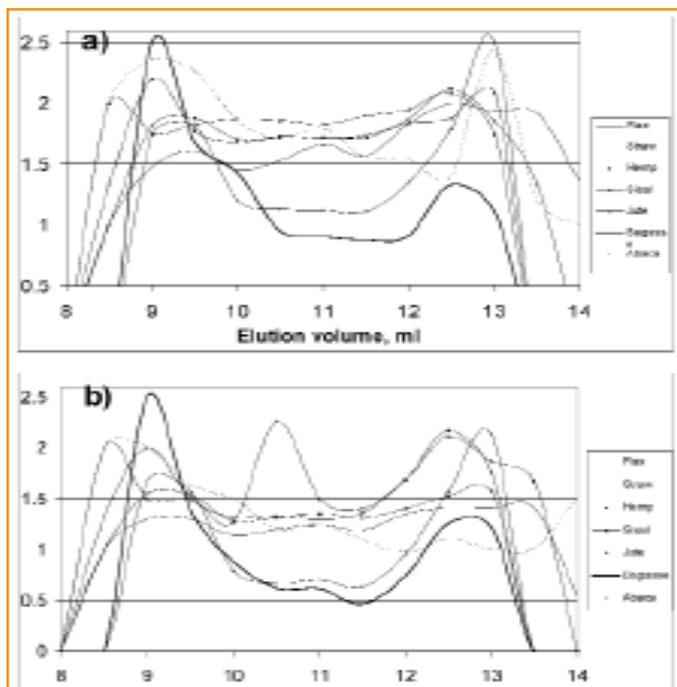


Fig. 3 - The 240 (a) and 260 (b) CCD plots of non wood soda lignins.

radicals in accordance with procedure described elsewhere [10].
6) Lignin bioactivity was assessed using test with bacteria *Escherichia coli*, performed as described previously [11].

Results and discussion

The multi-modal patterns of UV₂₈₂ and RI chromatograms presented in the Figure 1 indicated the complexity of non-wood soda lignins composition. It could be noted that Jourdi GEL GBR Mixed bed linear column working in organic solutions, acidic and alkaline media may be considered as a relevant tool for lignin analysis by SEC, although under alkaline solutions this column is not well investigated yet. The absence of strong concentration effects under experimental conditions allows to attribute the separation to quasi exclusion one. This column is non-electroneutral. Due to its ionogenic properties conjugated lignin structures are more ionized, that lead to obtaining of multi modal chromatograms and, respectively, allows to characterize the lignin chemical heterogeneity in details.

For all soda lignins investigated, five peaks (I, II, III, IV and V) were found under SEC region ($V_o < V_e < V_f$), and one peak (VI) eluted in the adsorption region of the column ($V_e > V_f$). The concentration of macromolecules eluted in the peak IV was, according to RI-chromatogram, the highest. However, insignificant absorbance at

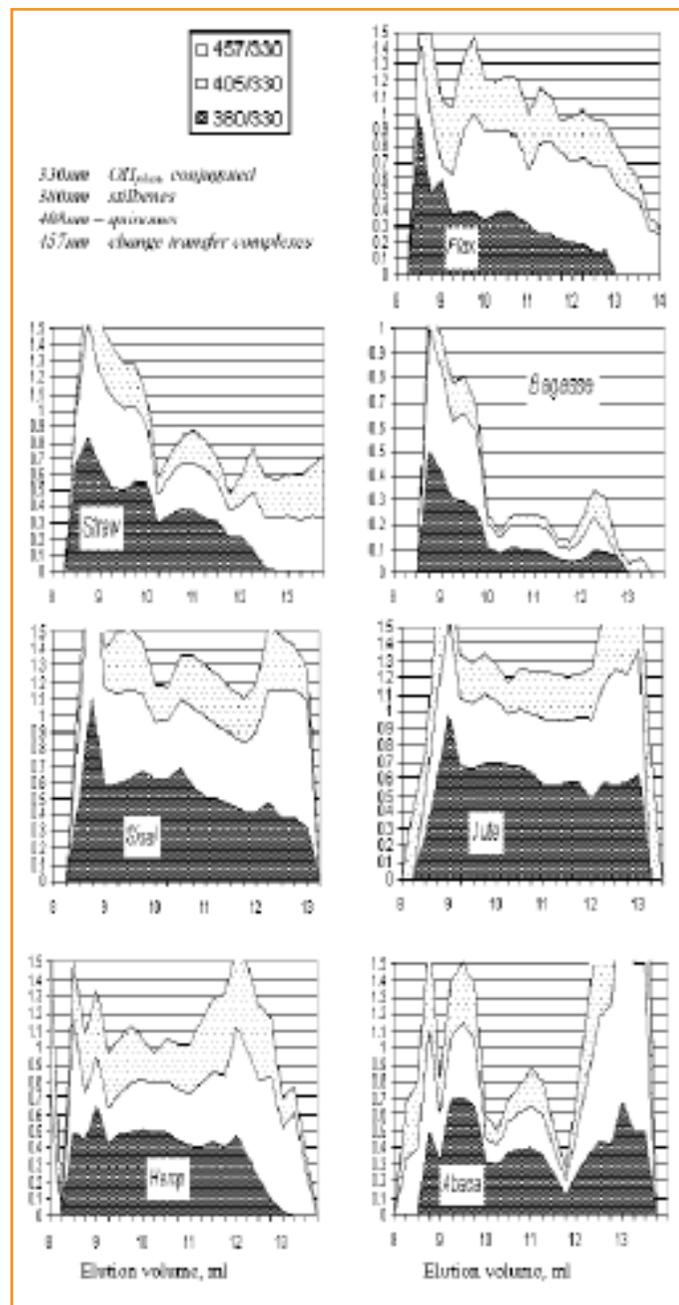


Fig. 4 - Conjugated structure distribution attributed to MMD for soda lignins

282 nm indicated negligible concentration of lignin in this fraction. In contrast, for all samples, the peak I that was negligible by concentration according to RI-response strongly absorbs at 282 nm. At the same time, for lignins of abaca and bagasse, in comparison with other samples, an extremely high absorbance was observed in the peak III. The significant difference between UV

and RI responses practically for all lignins reveals their chemical heterogeneity attributed to MWD.

To characterize the chemical structure of lignin fractions differed by MW, the UV-spectra in peaks I-IV (Fig. 1) were calculated on the basis of the UV-chromatograms and RI-chromatograms, utilizing the UV:RI ratio. The magnitude of UV_{λ}/RI responses represents the absorbance value at wavelength λ per mass of substance eluted at current elution volume V_e ; thereby, it is equal to absorptivity. The calculated UV-spectra of fractions corresponding to peaks I-IV (Fig. 2) revealed the quantitative and qualitative differences in chemical composition attributed to MWD between the lignins under study (Fig. 2).

The absorbance at 280-290, 310-360, 370-390 and 400-440 nm are due to substituted benzene rings, ionized conjugated phenols, stilbenes and quinones, respectively, presented in lignin structure [12-14]. At the same time absorbance around 245 nm may be related both to lignin and oxygen-containing heterocycles of furan and/or pyron-type in the samples [15, 16]. The problems regarding the attribution of the absorbance above 280 nm exclusively to lignin or heteroaromatics was discussed elsewhere [17]. During pulping there is a possibility of grafting of heteroaromatic cycles containing intermediates/products of destruction of polysaccharides (mainly xylan and arabinogalactan) on lignin macromolecules. An adequate technique of identi-

fication and quantification of heteroaromatics impurities in lignin requires further elaboration. However recently it has been shown that for lignin the ratio of A240/A290 or A260/A290 is lower than 1.2 [18]. The lowest heteroaromatic impurities were observed in soda lignin of bagasse, as these were found only in the high MW region (peak I, Fig. 2.) For all samples, heteroaromatic impurities were found in the peak I and, apart from abaca and bagasse, in the peak III. In the case of the hemp and sisal, these were found in all SEC peaks (I-IV) (Fig. 2). The 240 CCD and 260 CCD plots presented in Fig. 3 demonstrate a non-uniform distribution of heteroaromatics over MWD for all lignin samples under study (Fig. 3).

A family of CCD curves (A_{λ}/A_{330}) shown in Fig. 4 reveals distribution of conjugated structures attributed to MWD. For all lignins investigated, the range of highest MW is enriched with conjugated structures - quinones and stilbenes (405 CCD and 380 CCD, respectively). The lowest content of stilbenes, quinones and charge transfer complexes (CTC) is observed for bagasse lignin. Flax, sisal and jute lignins are characterized with highest contents and more uniform distribution of quinones and stilbenes over MWD. For all soda lignins, the distribution of CTC (457 CCD) over MWD is similar to that of quinones. This could be connected with the acceptor role of quinone substructures in lignin charge transfer autocomplexes.

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Characterization of non-wood soda lignins

Lignin sample	OH phen, %		Development of π -conjugation		RSC* systems	Suppression of bacteria <i>Escherichia coli</i> development, %
	Total	Weakly acid	Mean conjugation length, number of CH fragments	Number of polyconjugated clusters per 1,000 PPU		
Flax	2.57	0.31	30	3.0	1.0	99.2
Straw	2.20	0.51	25	1.5	0.2	13.0
Hemp	2.06	0.20	15	1.8	0.3	41.0
Sisal	3.18	0.26	17	1.0	1.25	96.0
Jute	3.34	0.18	12	1.5	1.25	15.0
Abaca	4.28	0.26	12	1.5	1.0	88.0
Bagasse	3.04	0.05	10	0.7	0.4	28.0

*RSC was defined as an inverse value of EC_{50} . EC_{50} is the concentration of the lignin samples needed to decrease the initial DPPH concentration by 50%

Summarizing, it could be concluded, that chemical composition distribution (CCD) curves, obtained using multi-wave UV/Vis detection at SEC analysis, clearly demonstrate the considerable differences between non-wood soda lignins investigated both in the content and localization of conjugated phenolic structures attributed to MWD.

These results gave an opportunity to explain up to definite degree the distinctions in chemical reactivity and bioactivity detected earlier for these samples [10, 11]. Thus, jute, sisal and flax lignins which, as mentioned above, have a highest content and lower heterogeneity of polyconjugated substructures distribution reveal themselves as good radical scavengers although ESR spectroscopy show significant differences in integrated parameters of conjugation development of these lignins (Table). At the same time, straw and flax lignins, according ESR and differential UV/Vis spectroscopies, are characterized by close parameters of the polyconjugated and condensed substructures development strongly influencing antioxidant and biological activities of lignin (Table). In spite of the similarity of the above men-

tioned parameters for the flax and straw lignins, the data of radicals scavenging activity towards DPPH and bioactivity towards development of bacteria *Escherichia coli* demonstrate the lower activity of straw lignin as compared with the flax one. The SEC data, which revealed the details in the localization of the conjugated structures over MWD of this samples (Fig. 4) allowed to explain these facts.

Conclusions

The results obtained have shown that the multiwave UV/Vis-SEC analysis and chemical composition distribution (CCD) curves, calculated on its basis are useful analytical tools for the characterization of the lignin chemical heterogeneity attributed to the MWD. This approach can be considered as the only possible method for characterization of highly reactive conjugated substructures distribution over fractions differing by MW without their isolation.

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Rivelazione UV/visibile multifrequenza in analisi di SEC per la valutazione di siti reattivi e sottostrutture policoniugate nelle distribuzioni di PM delle lignine

ABSTRACT 

È stato proposto un metodo per la determinazione dell'eterogeneità chimica della lignina. Esso è basato sul calcolo delle curve di distribuzione della composizione chimica da dati ottenuti mediante rivelazione UV/vis durante analisi SEC. L'applicabilità del metodo sviluppato è stata dimostrata dallo studio di un set di lignine alcaline non contenenti zolfo. Sono state rivelate differenze significative sia nel contenuto sia nella localizzazione delle strutture fenoliche coniugate ed eteroaromatiche per le lignine in studio.