Developing new methodology to determine changes to the lignin macromolecule during delignification

Norman G. Lewis and Thomas L. Eberhardt
Associate Professor and Graduate Student, Dept. of Forest Products, Virginia Tech, Blacksburg, Va. 24061.

Corinne E. Luthe
Research Scientist, Paprican, 540 St. John's Boulevard, Point Claire, Que., Canada

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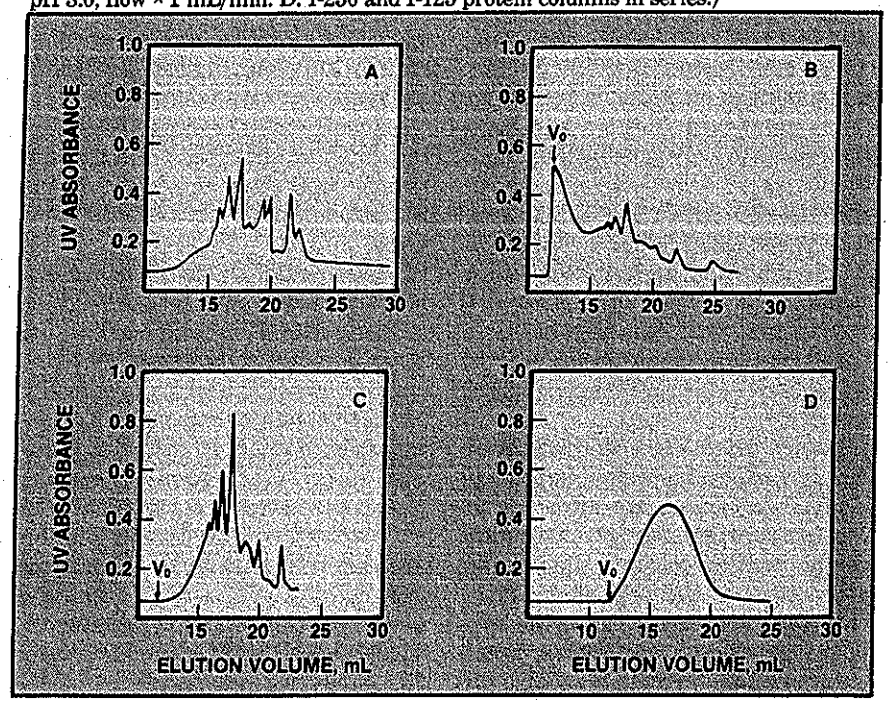
Lignin, a widely abundant, complex, terrestrial plant polymer can readily be removed from cell walls by sulfonation. Such treatment affords a complex mixture of aqueous soluble sulfonated lignins. Surprisingly, 80% of the lignosulfonates from spruce wood (*Picea abies*) consist of an homologous series containing identical repeating units (*1*).

Interestingly, structures have been put forward but without spectroscopic evidence to support them. The remainder of these soluble lignins (about 20%), collectively referred to as "hemilignins" (*1*), were purportedly of a lower weight-average molecular weight (about 2000). However, no structures have been reported for these substances.

It has been suggested that both lignin sulfonation and condensation reactions occur during sulfite-promoted delignification, with sulfonation predominating (*2*). It is difficult to estimate the extent of such condensation reactions, because the chemical nature of the native lignin polymer in plant tissue (*in situ*) has never been established.

New approaches are required to define both the chemical nature of native lignin and the changes the macromolecule undergoes during its removal from plant tissue. Here, we describe recent progress in this complex issue.

1. Chromatograms of dissolved UV-absorbing materials in various spent sulfite liquors. A: high-yield, softwood. B: low-yield, softwood. C: low-yield, filtrate through a 10,000 molecular weight cut-off membrane. D: low-yield, retentate. (A–C: HPLC columns, Waters I-125 and 60 protein columns in series and eluted with 50-mM citric acid/Na_2HPO_4 buffer, pH 3.0, flow × 1 mL/min. D: I-250 and I-125 protein columns in series.)



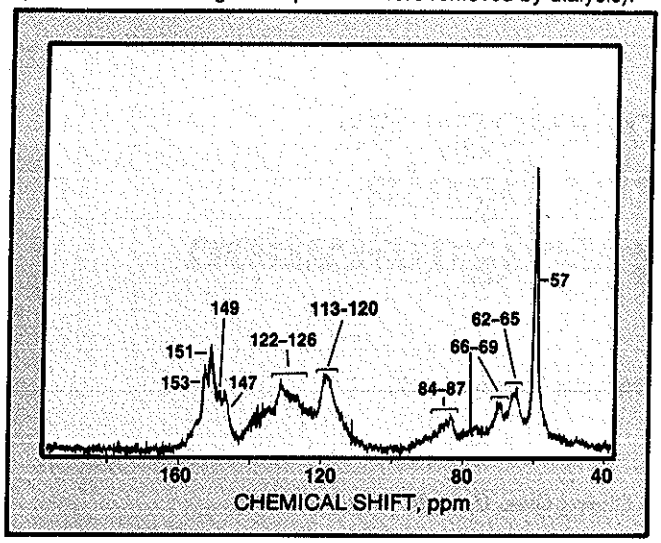
Results and discussion

Figs. 1A and 1B show typical high-performance liquid chromatograms for spent sulfite liquors (SSL) from both high- and low-yield pulping processes. In addition, the effect of passage of a low-yield softwood SSL through a membrane (with a nominal molecular weight cut off of 10,000) can be seen in **Figs. 1C and 1D**, representing the filtrate and the retentate, respectively.

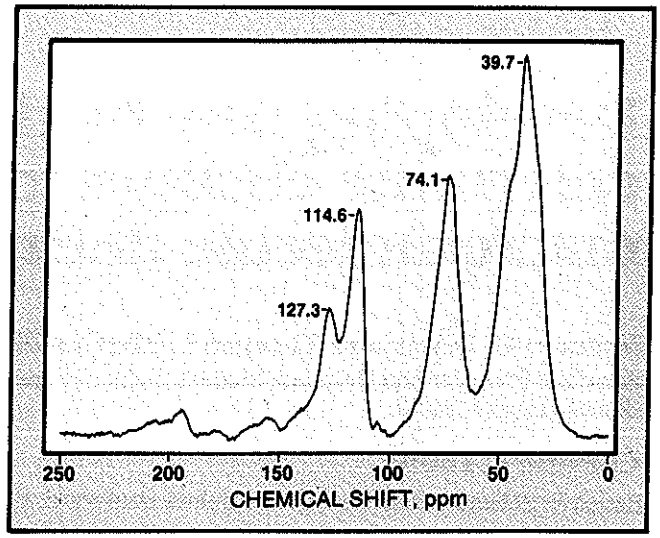
In previous experiments (*3*), using a continuous flow delignification apparatus, the solubilized lignin sulfonates from both spruce (*Picea mariana*) and aspen (*Populus tremuloides*) were analyzed at various intervals using high-performance liquid chromatographic (HPLC) techniques. It was observed that, during the early stages of lignin removal (<40%), only lignosulfonates of low molecular weight (paucidisperse) were solubilized. These substances are the so-called "hemilignins."

In an attempt to identify these materials, we evaluated different methods

2. ^{13}C NMR spectrum of polydisperse, low-yield lignosulfonate. (Low-molecular-weight components were removed by dialysis).



3. Solid-state ^{13}C NMR difference spectra of *T. aestivum* with β-^{13}C ferulic acid administered.



for their isolation and characterization. To date, the most efficient method developed was to first convert the sodium salts of the lignosulfonates to their free acid (H^+) forms, methylate with diazomethane, and purify the sulfonomethyl derivatives by using HPLC techniques. In this way, about 40 compounds were isolated and identified (*3, 4*). All were C_9–C_{11} monomers with different extents of sulfonation on the propanoid side chain. No dimers or oligomers of higher molecular weight were isolated. These results thus suggest that "hemilignins" are indeed monomeric and do not have a molecular weight of 2000, as intimated previously (*1*).

Having determined the nature of these low-molecular-weight lignin sulfonates, we focused our attention on the more polydisperse high-molecular-weight fraction released during the later stages of delignification (as in Fig. 1D for instance). Unlike the low-molecular-weight constituents, there are apparently no distinguishable (or distinct) moieties. **Figure 2** shows a typical ^{13}C NMR spectrum for a dialyzed polydisperse lignosulfonate retentate from spruce wood. The resonances can be tentatively assigned (using DEPT pulse sequences) as follows:

- δ 57 for OCH_3
- δ 62–65 for Cγ, CH_2
- δ 66–69 for CH, CH–OH
- δ 84–87 for C_β, CHOAR
- δ 113–126 for C-2, C-5, and C-6
- δ 128–130 for C-1
- δ 147–153 for C-3 and C-4.

This pulse sequence shows that most of the resonances between 122 ppm and 153 ppm were due to quaternary carbon atoms; indeed, the broad (quaternary) resonances from 120 ppm to 140 ppm suggest condensation reactions have taken place.

However, such spectra provide us with little exact information on the changes that the macromolecule has undergone during its removal from wood.

Consequently, we developed an approach to determine first the bonding environments of specific carbons in lignin in intact plant tissue. This was achieved by administering specifically labelled ^{13}C lignin precursors to growing intact plants over extended durations of time (*5*). **Figure 3** shows the ^{13}C enhanced resonances in plant tissue (*T. aestivum*) with previously administered β-^{13}C ferulic acid. Resonances were noted at δ 127.3 (for C_β, coniferyl alcohol-like structure), 114.6 (for C_β ferulate), and at 74.1 and 39.7 ppm. The latter two resonances are as yet unassigned. Having demonstrated that specific bonding arrangements can now be determined in plant tissue, we next isolated this lignin as its acetal derivative (*6*) to ascertain any significant bonding changes that the macromolecule may have undergone during this treatment. In this case, the solubilized lignin also showed enhanced resonances at δ 127–128 (broad), 115.5, 74.1, 38.91, and 36.2 ppm. In other words, the resonances were at values similar to those observed for the plant tissue itself. Thus, we are now able to examine the effects of conventional delignification treatments—e.g., sulfite pulping on the lignin macromolecule.

Conclusions

It now appears possible to determine the changes in bonding patterns of specific carbons during lignin removal. This now allows us to examine the process of delignification in a much more rational manner.

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