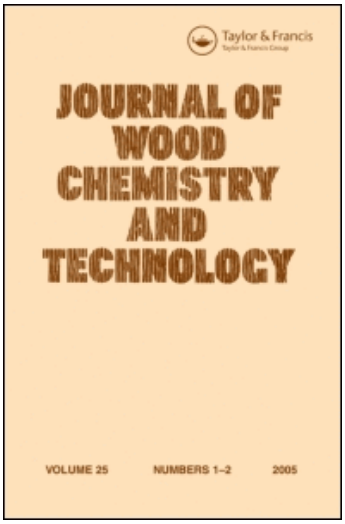

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David M. Hanson[a]; Donna Cietek Wenzel[a]
[a] International Paper Company Corporate Research Center, Tuxedo Park, New York

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DEVELOPMENT OF SIZE EXCLUSION CHROMATOGRAPHY/LASER INDUCED FLUORESCENCE ANALYSIS OF ISOLATED LIGNINS

David M. Hanson and Donna Cietek Wenzel
International Paper Company
Corporate Research Center
Tuxedo Park, New York 10987

ABSTRACT

Size Exclusion Chromatography/Low Angle Laser Light Scattering (SEC/LALLS) has been used to monitor changes in the molecular weight disbribution (MWD) of lignins recovered from alkaline pulping liquors. While developing a method of molecular weight analysis, we discovered that fluorescence was contributing to the detected signal. During the course of the experiments, we realized that this laser-induced fluorescence, could be used to monitor lignin. We modified the filters in the LALLS instrument and sucessfully measured lignin fluorescent properties. We found that fluorescence of lignin increased during the course of pulping with the formation of fluorescent structures excited at the wavelength of the laser (632.8 nm) being uniform throughout the molecular weight range.

INTRODUCTION

In pulping liquor, lignin can undergo a number of changes, most of which involve the breaking of linkages and condensation reactions. To examine the nature of lignin, there are several instrumental techniques: infrared spectroscopy, ultraviolet spectroscopy, and nuclear magnetic resonance spectroscopy, as well as coupled analyses such as pyrolysis/gas chromatography/mass

spectroscopy and size exclusion chromatography/low angle laser light scattering (SEC/LALLS)[1].

Size exclusion chromatography (SEC) is a technique that separates the molecular species of a macromolecule according to hydrodynamic volume. It produces a distribution of molecular weight as a function of solvent volume (time). However, the molecular weights obtained are only apparent, since they are based on external calibration using well-characterized narrow distribution standards of different polymers. The addition of a LALLS photometer allows for calculation of absolute molecular weights. This approach has been used to measure molecular weight distributions of celluloses via their tricarbanilate derivatives[2].

During the LALLS experiments on lignin, we found that fluorescence/phosphorescence in the scattering cell complicated measurements. (In this paper, fluorescence will refer to both fluorescence and phosphorescence phenomena.) Bublitz[3-5] and Lundquist[6-7] have published a number of papers on the fluorescence of lignin. Most of their work involved excitation at 280 to 315 nm for a maximum peak intensity. Since the laser line used in our instrument was so far removed from the excitation maxima, we did not expect the fluorescence we observed. Therefore, the occurrence of this fluorescence with excitation at high wavelength is interesting as a possible technique for studying lignin.

Fluorescence spectra obtained on a Perkin Elmer Model 650-10S fluorescence spectrophotometer showed no measurable fluorescence when the excitation wavelength was 632.8 nm, the same wavelength as the LALLS laser. At 632.8 nm, the laser is appreciably more powerful than the xenon lamp used in the Model 650-10S fluorescence spectrophotometer. Therefore, we assume that the 2.0 mW HeNe laser used in the LALLS photometer can induce fluorescence in lignin measurable above the instrument's dark current.

Fluorescence at long wavelengths often implies a considerable amount of conjugation in a molecule. Therefore, if the high light intensity provided by the laser enables us to measure the fluorescence of lignin we may be able to examine conjugation in lignins isolated from pulping liquors.

RESULTS AND DISCUSSIONS

Experiments using the interference filter on low kappa kraft lignin demonstrated that as expected with size exclusion chromatography, molecular weight decreased with increasing elution volume. Figure 1 shows the low kappa kraft lignin light scattering signal from the KMX-6 Low Angle Laser Light Scattering (LALLS) photometer and the Differential Refractive Index (DRI) response, normalized by the computer to the same height. Although high and low kappa kraft lignins were examined throughout this study, for illustrative purposes we will discuss only low kappa kraft lignin results. Note that the LALLS response increases with increasing molecular weight of the material -- a classic light scattering response.

When we analyzed the same low kappa kraft lignin using no filter after the scattering cell, we got the result shown in Figure 2. In this case, the KMX-6 response is composed of both fluorescence and light scattering. Note that the response is much broader and has a shoulder at the high MW end.

Figure 3 demonstrates the response observed on the KMX-6 when an RG-665 Schott glass filter was incorporated into the SEC/LALLS system. The LALLS photometer, in this case, will only detect fluorescence of the lignins. The similarity of the DRI and fluorescence curves in this sample indicates that changes in molecular weight causes little, if any, change in fluorescence.

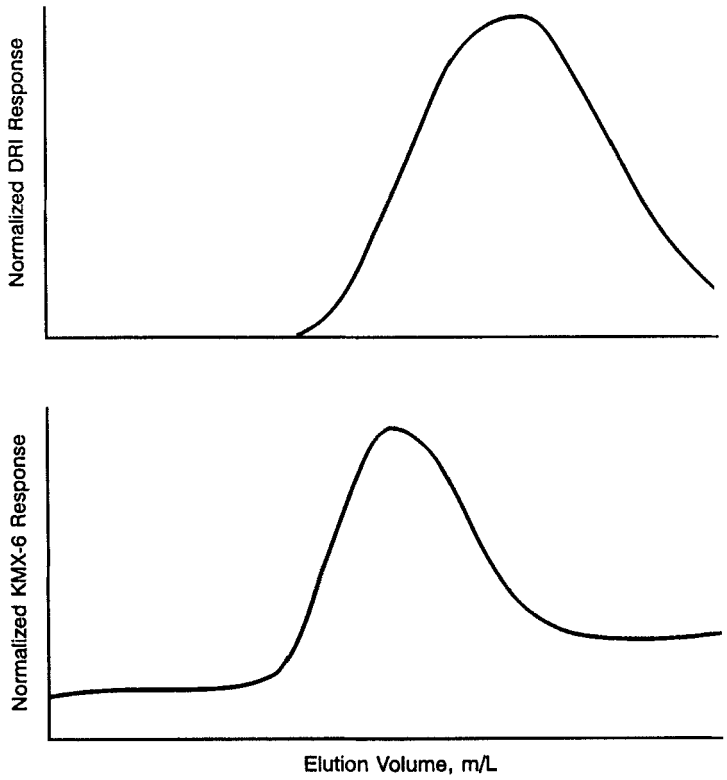

FIGURE 1. LOW KAPPA KRAFT - INTERFERENCE FILTER IN PLACE - LIGHT SCATTERING ONLY

We used the DRI and fluorescence mode with the RG-665 filter to analyze samples of the lignin. The DRI response gives a concentration response, the KMX-6 signal, a fluorescence intensity. By normalizing the fluorescence signal via the fluorescence/DRI response, we removed concentration differences between samples.

Plots of pulping time versus fluorescence/DRI response for softwoods and hardwoods are given in Figures 4 and 5. The lignin shows a general increase in fluorescence during the course of the

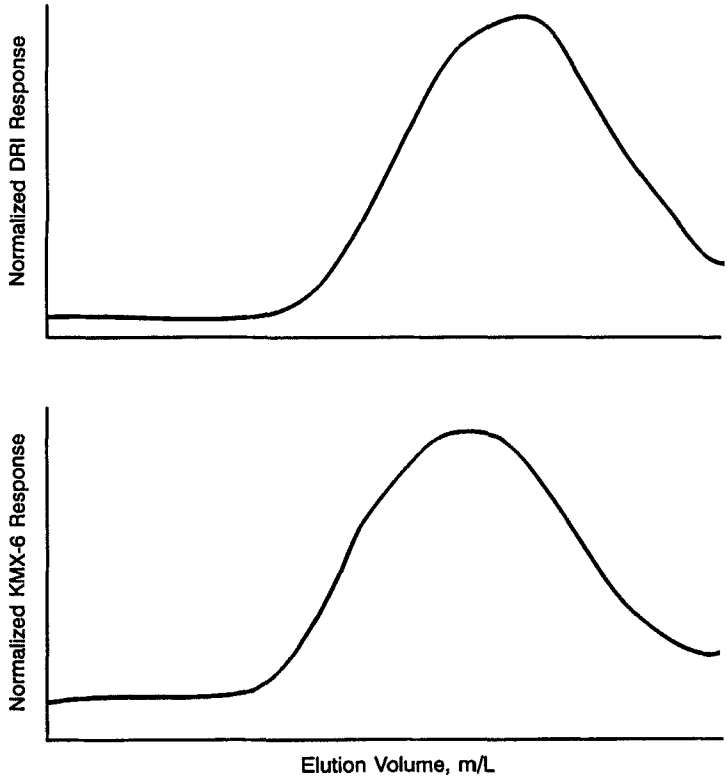

FIGURE 2. LOW KAPPA KRAFT LIGNIN - NO FILTER - LIGHT SCATTERING AND FLUORESCENCE

cook. This intensity increase is greater for softwoods than for the hardwood lignins. Fluorescence of samples in the higher wavelength regions normally can be associated with an increase in the amount of conjugation present in the molecule. These lignins from both hardwood and softwood cooks behave as past studies would predict; that is, more condensed lignin structures form during the course of pulping.

In summary, this study has shown that laser-induced fluorescence of lignins at 632.8 nm can be measured with minor modifica-

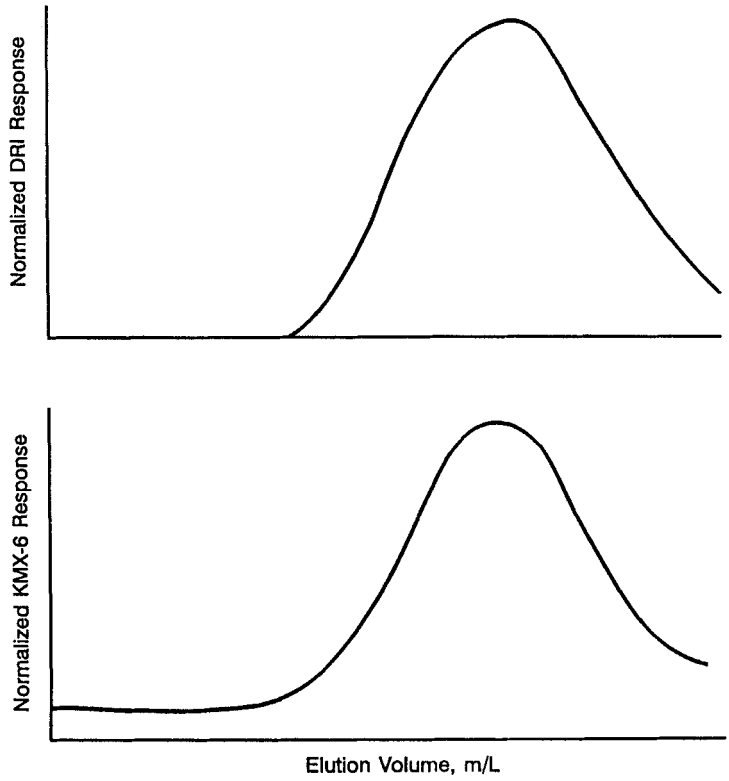

FIGURE 3. LOW KAPPA KRAFT LIGNIN - RG665 FILTER - FLUORESCENCE

tions to the existing LALLS photometer. Furthermore, kraft lignins can be analyzed using a differential refractive index detector as a concentration detector and the LALLS unit as a fluorescence detector. Both softwood and hardwood lignins show an increase in fluorescence per unit concentration during the course of the pulping reaction. Since changes in fluorescence can be related, in theory, to extension of conjugation within the lignin molecule, the measurement of laser induced fluorescence of lignins may be a useful tool in the investigation of lignin reactions in pulping systems.

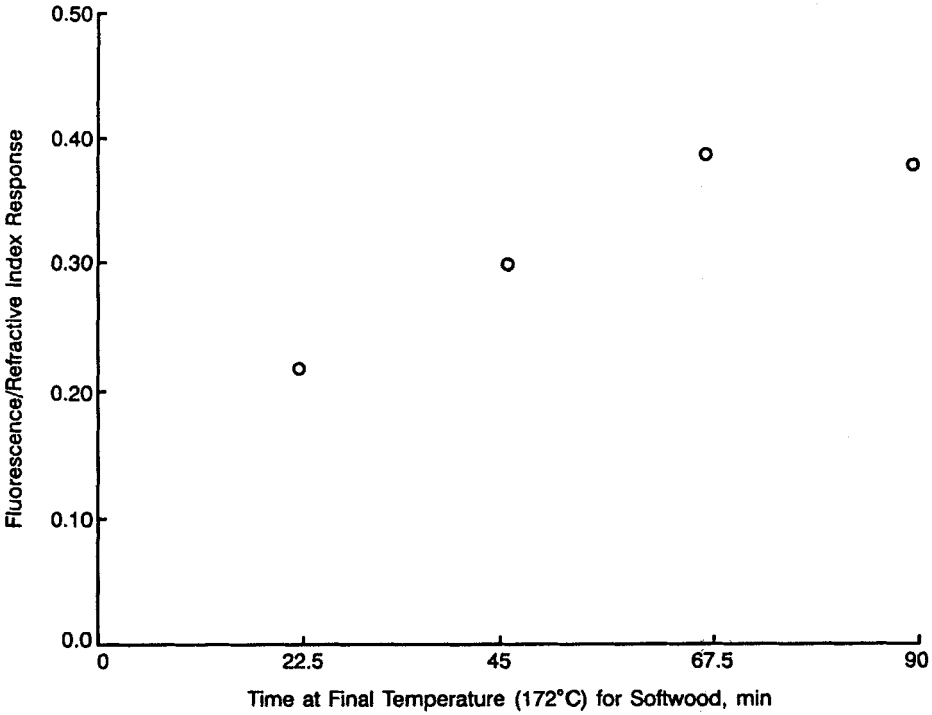

FIGURE 4. FLUORESCENCE/DRI RESPONSE VERSUS SAMPLING TIME IN A SOFTWOOD COOK

EXPERIMENTAL

A schematic of the SEC/LALLS system used in our laboratory appears in Figure 6. A Waters Associates Model ALC-201 high performance liquid chromatograph incorporating μ-styragel columns, connected in series, is interfaced to a Chromatix KMX-6 LALLS photometer, which uses polarized radiation from a 2.0 mW HeNe laser at 632.8 nm. We measured the concentration of the eluting solute after exiting from the LALLS flow-cell by incorporating an ultraviolet (UV) or differential refractive index (DRI) detector. A Chromatix LDS-2 laboratory data system digitizes the analog

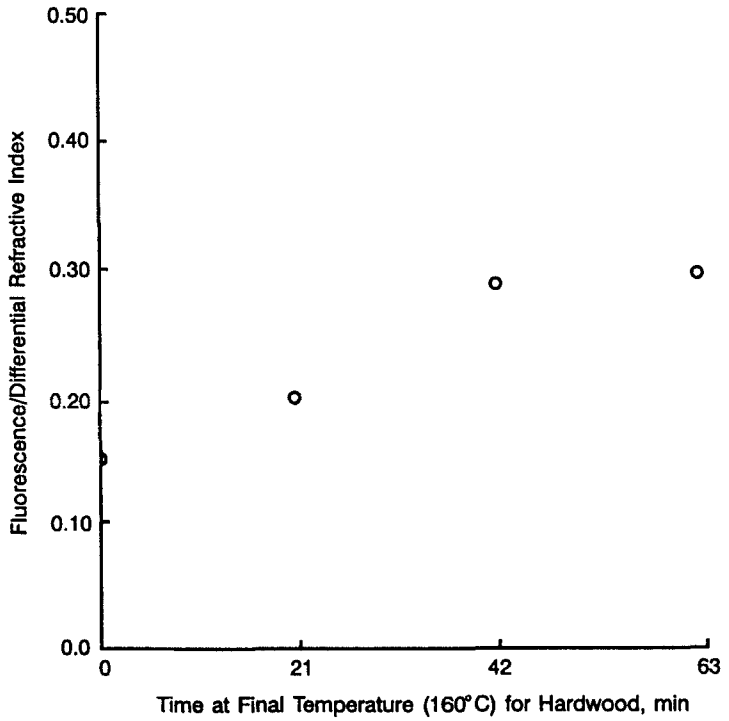

FIGURE 5. FLUORESCENCE/DRI RESPONSE VERSUS SAMPLING TIME IN A HARDWOOD COOK

signals from both the LALLS and the concentration detectors for on-line data acquisition and analysis. In the ideal case, the signals seen by these detectors are all due to concentration and scatter of laser light.

The KMX-6 optics are fixed with an allowance for minor adjustments necessary for fine alignment, with the exception of the filter holder and analyzing polarizer. These are removable since they are not used in normal operation. The analyzing polarizer was removed from the LALLS photometer for these studies. The filter holder allows placement of any 2 x 2 in. filter in the instrument

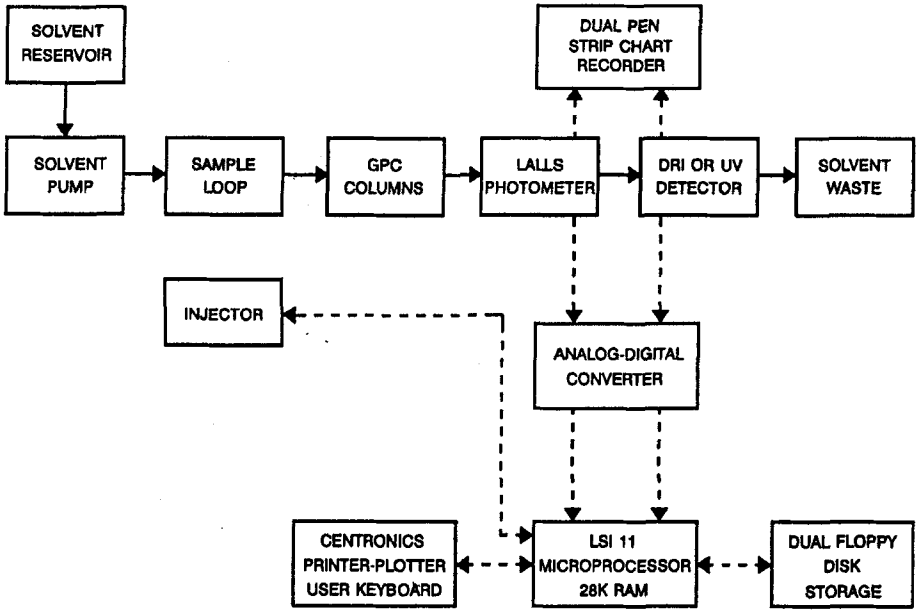

FIGURE 6. SCHEMATIC OF LALLS/SEC UNIT

after the scattering cell. When no filter is present, all the scattered light within the angle measured is collected and sensed by the photomultiplier tube (PMT). This light can consist of scattered light, fluorescence, phosphorescence, and Raman lines, if present.

Placement of an interference filter in the holder allows light of the laser wavelength (632.8 nm ± 2.0 nm) to pass to the photomultiplier while excluding other bands. Normally this filter is used to remove unwanted phenomena such as fluorescence, phosphorescence, and Raman scatter from the sample.

Fluorescence can be observed by using a cut-on filter made of Schott glass. We used RG-665 and RG-695 filter for these experiments; their spectral characteristics are shown in Figure 7. These

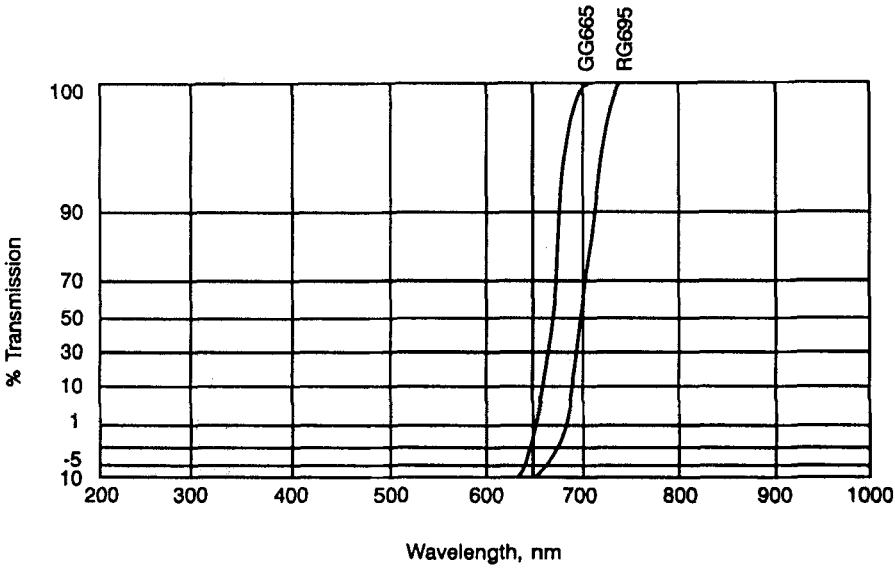

FIGURE 7. TRANSMISSION SPECTRA OF BAND PASS FILTERS

filters eliminate the laser wavelength, while allowing the passage of higher wavelengths produced by fluorescence and phosphorescence. All normal light scattering settings and optimization were used while observing fluorescence, with the exception of the PMT setting, which was adjusted to a constant voltage.

Raman scatter is normally a very weak signal. We used an Ealing 35-5396 short pass filter to investigate any possible Raman contribution and found no detectable signal.

We performed the pulping studies on white pine and southern pine, using laboratory-scale digesters. We used the kraft process to prepare samples. The black liquors obtained from the cooks were precipitated by addition of sulfuric acid to pH 2. They were then centrifuged, washed, and dried. The dry lignins were dissolved at

TABLE 1. Lignin Isolation

- Softwood

  90 min to 172°C
  90 min at 172°C
  16% active alkali

- Hardwood

  60 min to 160°C
  85 min to 160°C
  16% active alkali

- 5 sample intervals during the cook

- Isolate by acid treatment

  pH 2 insolubles washed well to remove inorganics

a concentration of 0.2% (wt/vol) in HPLC grade tetrahydrofuran (THF) and then filtered through a 0.45 μ Millex-HV disposable filter. A 1.0 ml sample volume was injected into the SEC/LALLS system, which contained three μ-styragel columns (1000,500,100 Angstroms) and a differential refractive index detector for monitoring concentration. The mobile phase consisted of HPLC grade THF pumped at a flow rate of 1.0 mL/min. All experiments were performed at ambient temperature.

To examine changes in lignin during pulping, we removed black liquor samples from kraft cooks of hardwood and softwood. The cooks were done according to the conditions listed on Table 1. Because hardwoods and softwoods pulp at different rates, we collected the samples for analysis at different intervals in the cook.

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