# Kerr gated resonance raman spectroscopy in the studies of lignin polymerization

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## Introduction

Lignins are phenolic polymers that form in cell walls of terrestrial plants. After cellulose, they are the second most abundant biopolymer in nature, providing strength and facilitating water transport in land plants. They are synthesized *in vivo* from three different types of phenolic monomers. The structures and names of these three *p*-hydroxycinnamyl alcohols are given in Figure 1.

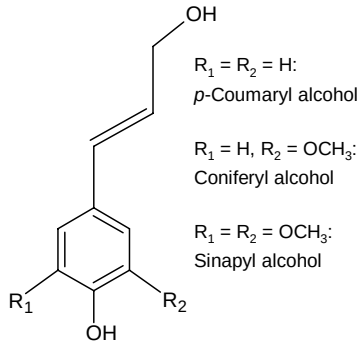


**Figure 1.** Structures of the three lignin precursors.

The result is a very complex branched plant polymer composed of phenolic and non-phenolic inter-linked units. Therefore it contains dozens of different inter-unit bond types. The polymerization of the monomeric precursors by random coupling reactions can not be studied *in vivo*, which is why so much effort has been done in synthesizing lignin *in vitro* under conditions that resemble those in nature. Therefore many theories on lignin structure and biosynthesis rely upon *in vitro* experiments on the polymerization of the precursors. *In vitro* synthesized lignin is generated by enzyme-mediated radical-radical coupling of the precursor. The most common natural lignin precursor is coniferyl alcohol[1)].

Spectroscopy, in all its forms, provides a powerful tool for the study of structure. In polymer science spectroscopic methods provide important means of identification, characterizing microstructures, orientation, intermolecular interactions etc. A great advantage of Raman spectroscopy is that it (most often) does not require sample preparation, but the samples can be measured directly. Another advantage is that it is a comparatively fast method of analysis; a Raman spectrum can be obtained within a few minutes. However, Raman spectroscopy often suffers from the disturbing interference of fluorescence, either from the sample itself or from minor amounts of impurities, which can undermine the signals from the sample. This can be avoided using a laser with a wavelength in the NIR-region, using surface-enhanced resonance Raman scattering, or using, as in the present case, Kerr gated Raman spectroscopy. Lignin being exceedingly fluorescent, limits the use of Raman spectroscopy to the three previous mentioned methods. The reason for choosing the Kerr gated resonance Raman method is due to the benefit of the resonance effect of the lignin sample as well as the fluorescent rejection. Recent studies on enzymatic *in vitro* oxidation of wood fibers have shown them to contain long-lived lignin radicals[2)], which may be detected by resonance Raman spectroscopy.

The objective of this study was to be able to make direct measurements on a lignin polymerization solution. This was done by slowly polymerizing the lignin, and letting the solution being pumped through a quartz flow cell before returning to the flask. We were thereby able to follow *in situ* the development of lignin as it is being polymerized using Kerr gated Raman spectra. Another idea was to examine the structural impact of the polymerization environment, by doing this experiment in a solution containing pectin or cellulose. This has previously been studied by Cathala and Monties[3)]. Our additional goal was to monitor the signals from coniferyl alcohol radicals and lignin radicals.

## Experimental

The experimental setup is shown in Figure 2. The lignin polymerization was performed in beaker C.

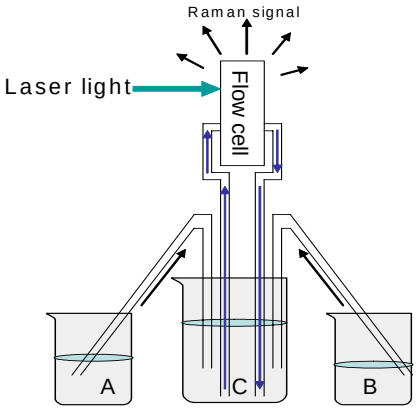


**Figure 2.** Experimental setup.

Beaker A contained coniferyl alcohol 0.08 M in a solution of 44 % ethanol and 56 % buffer (citric acid phosphate 0.1 M, pH 3.5).

Beaker B contained hydrogen peroxide (0.16 M) in the same buffer.

The polymerization was initiated as the solutions from beaker A and B were pumped slowly at pumping rates of approximately 2 ml/h into beaker C, where the polymerization took place.

Beaker C contained initially always 80 % buffer, 20 % ethanol and Horse Radish Peroxidase (from Sigma-Aldrich: 1500 u, supplier units). Beside this, varying amount of cellulose or pectin was added (in different experiments) to investigate the influence of their presence. The ethanol: buffer (1:4) ratio was thus held constant during polymerization. The Figure of 44% (instead of 40 %) ethanol in beaker A was derived by correcting for a minor pumping rate difference from beaker A and B.

The polymerization was stopped when adding ascorbic acid, and the lignin formed was dissolved when NaOH was added, causing the solution to become alkaline, and the phenolic groups to ionize. Raman spectra were then obtained from these alkaline solutions.

The laser equipment used for the measurements provided 800 nm, 1 ps, 2-3 mJ fundamental pulses at 1 kHz repetition rate. The fundamental laser output was split into two beams; one part was taken for the gating pulse to drive the Kerr gate, and the remaining part was frequency doubled and used as a probe beam. The signal was obtained by averaging twenty cycles amounting to 600 s acquisition time. Further details of the setup can be found elsewhere[4].

**Results**

The influence on Raman spectra by variation in polymerization time, content of citrus pectin, sugar beet pulp pectin and cellulose was investigated. Previous reports on Kerr gated Raman spectroscopy on lignin has been given by Saariaho *et al.*[5]. They reported that both lignin and cellulose were detectable with an excitation length of 400 nm. A general overview on Raman spectroscopy as applied to lignocellulosic has been given by Agarwal[6].

In a series of measurements, lignin was polymerized without cellulose present, or with a content of cellulose of 3 %, 10 % and 30 %, respectively, suspended in the solution.

In Figure 3 the four Raman spectra of these lignin polymerizations are shown:

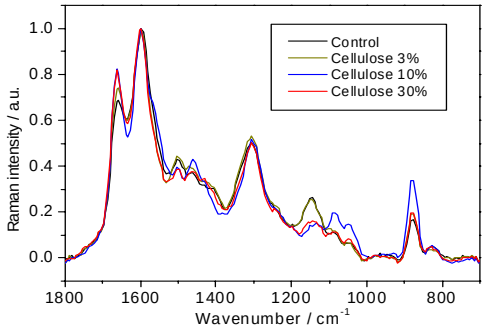


**Figure 3.** The Raman spectra obtained from four different lignin polymerizations: A control sample with no cellulose and three samples with 3 %, 10 % and 30 % cellulose, respectively. All four samples were polymerized during three hours. The spectra are normalized with respect to the band at approximately 1600 $cm^{-1}$.

From Figure 3 it is observed that the different amount of cellulose has an influence on the Raman spectra of the lignin creation. This is particularly clear in the area between 1000 and 1200 $cm^{-1}$. Beside this the intensity ratio of the two bands at approximately 1660 $cm^{-1}$ and 1600 $cm^{-1}$ change when adding more cellulose to the lignin creation beaker. The ratio (1660 $cm^{-1}$/1600 $cm^{-1}$) increases as the content of cellulose becomes higher. The band at 1660 $cm^{-1}$ is assigned to the double bond in conjugation with the aromatic ring from the lignin groups and the coniferyl alcohol. Cellulose has a strong band near 1100 $cm^{-1}$. This is not apparent from Figure 3. One would expect this band to be more intense when a solution of 30 % cellulose was added, than the solutions with a lower content of cellulose. This is not the case, and may be due to the increased influence of laser light scattering away from the solution, such that Raman signals originate only from the solution close to the beaker surface.

This is, however, also the case for the coniferyl alcohol and lignin. The insignificant cellulose signal is therefore ascribed to the relatively much stronger signals from coniferyl alcohol and lignin, which are resonantly enhanced when using an excitation wavelength of 400 nm.

In Figure 4 the Raman spectra of lignin creations containing citrus pectin (0.3 %) or cellulose (30 %), and a creation without pectin or cellulose are shown. The lignin creations have been synthesized over a time period of three hours. The spectra have not been normalized with respect to any band.

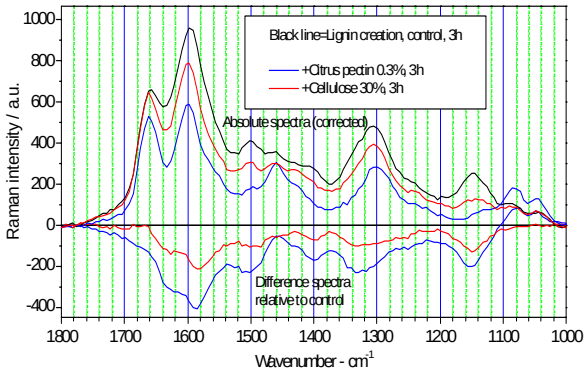


**Figure 4.** Top: The Raman spectra of lignin polymerization with no alteration (black), with 0.3 % citrus pectin (blue) and with 30 % cellulose (red). All three spectra were obtained after a three hours of polymerization. Bottom: Difference spectra obtained relative to the control spectrum.

From Figure 4 it is observed that the lignin polymerization containing citrus pectin is somewhat different than the polymerization without pectin, or the polymerization with cellulose. The intensity ratio (1660 $cm^{-1}$/1600 $cm^{-1}$) is even larger for the pectin containing solution, where the ratio almost equals one. From the difference spectra obtained by subtracting the Raman spectrum of lignin with cellulose or with pectin with the control spectrum, three other bands appear. These depression bands are located at approximately 1150, 1340 and 1400 $cm^{-1}$. These three bands appear to be more pronounced in the spectrum of the pectin containing polymerized solution than in the spectrum of the lignin polymerized in the beaker containing cellulose. Two of the bands, at 1150 $cm^{-1}$ and at 1340 $cm^{-1}$, seem to shift to higher wavenumbers, when changing the lignin polymerization environment from cellulose to pectin. This indicates the influence of the pectin and cellulose on the structural development of lignin during a polymerization process.

**Conclusion**

The spectra obtained using Kerr gated Raman spectroscopies from different polymerizations of lignin in different environments were of good quality. A high signal to noise ratio was achieved, and most fluorescence was avoided. From the spectra we were able to see the different influences of citrus pectin, sugar beet pulp pectin and cellulose on the lignin polymerization process. These differences will be subject of further investigations.

**References**

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