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RAMAN MICROPROBE INVESTIGATION OF MOLECULAR STRUCTURE AND
ORGANIZATION IN THE NATIVE STATE OF WOODY TISSUE

Progress Report
for Period April 1, 1982-July 31, 1983

R. H. Atalla

The Institute of Paper Chemistry
Appleton, Wisconsin 54912

August 1983

Prepared for
THE U.S. DEPARTMENT OF ENERGY

MASTER

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ABSTRACT

The key findings since initiation of the program on April 1, 1982 are:

1. It is possible to acquire significant Raman spectral information from domains that are 1 to 3 μ m in diameter in sections of plant cell walls.
2. Polarized spectra are sensitive to orientation of the electric vector relative to the plane of the cell wall, indicating anisotropy of molecular organization with respect to the geometry of the cell.
3. Preliminary explorations of woody tissue from black spruce and loblolly pine suggest that, in most instances, the aromatic rings of lignin are preferentially oriented in the plane of the cell wall, as are the anhydroglucose rings of the cellulose chains.
4. Preliminary studies also suggest that there is significant variation in the ratio of lignin to polysaccharide in the cell wall.

In related studies, evidence of transverse anisotropy in the structure of ramie fibers has been detected. A spectrum of lignin in situ has been recorded by difference spectroscopy. Also, a dissertation has been completed on the vibrational spectra of lignin model compounds.

RAMAN MICROPROBE INVESTIGATION OF MOLECULAR STRUCTURE AND ORGANIZATION IN THE NATIVE STATE OF WOODY TISSUE

OVERVIEW

Since the initiation of the program in April of 1982, the work has focused on installation of the Raman microprobe system and making it operational, on demonstrating the feasibility of the experimental approach, and on exploratory studies of molecular organization and structural variability in selected samples of plant tissue. Preliminary accounts are presented in the following sections.

The first section, a preprint of a note by Agarwal and Atalla, describes our first explorations of molecular organization in cell walls of earlywood tissue from a sample of spruce. There are clear indications of orientation of both cellulose and lignin molecules relative to the plane of the cell wall. We are continuing this work to establish whether such order is local or relatively general in the cell wall.

The second section, a preprint of a note by DeAmico and Atalla, describes explorations of compositional variations in the cell walls of a sample of loblolly pine tissue as reflected in the relative intensities of characteristic lignin and cellulose bands. The variations between adjacent cells in the same annual ring were found to be greater than the variations within an individual cell. In addition, the variability within cross sections of individual cells was found to be greater than within longitudinal sections. This section also incorporates a brief outline of earlier work, by Atalla and Heimbach, in which the spectrum of lignin in situ was recorded from groundwood.

The third section is an excerpt from an internal report by James Wiley describing studies on the structure of Ramie fibers. It provides clear evidence of anisotropy in the organization of cellulose molecules in planes perpendicular to

the cell axis as well as in planes parallel to the axis. The results, however, are not readily explained by prior models of molecular organization. Our study of this matter continues.

The fourth section describes refinements in the operation of the microprobe as well as developments of new methods for sample preparation and mounting. Spectra routinely acquired now are improved by at least an order of magnitude over those acquired when the system first became operational or those acquired at the manufacturers laboratory.

In a separate but closely related study recently concluded at The Institute of Paper Chemistry, the vibrational spectra of lignin model compounds were investigated. The dissertation by Susan M. Ehrhardt focused on the vibrational spectra of vanillin and its related monosubstituted and disubstituted phenyl compounds. The results will facilitate interpretation of lignin bands in the microprobe spectra.

CHRONOLOGY

The grant period commenced on April 1, 1982. The microprobe system was initially installed on May 20, 1982. However, because of modifications required in the spectrometer, and defects in some of the components provided by the manufacturer, the system was not operational until mid-August of 1982. Post-doctoral fellow Umesh Agarwal joined the program in November 1 of 1982.

The need for significant improvement in the quality of the spectra was recognized by January of 1983. The successful realignment of the microprobe and spectrometer system, described in a subsequent section, was carried out over a 10-day period early in February of 1982. Some of the spectra reported in subsequent sections were recorded prior to the realignment of the instrument.

Post doctoral fellow Umesh Agarwal is fully supported under the present grant. Mr. Robert DeAmico carried out his work as part of the requirements for the Masters Degree from The Institute of Paper Chemistry. His work was carried out between August of 1982 and the spring of 1983. Mr. Wiley is currently a doctoral candidate in the Institute's program; his work continues. Support for both Mr. DeAmico and Mr. Wiley was provided from funds of The Institute of Paper Chemistry.

REFERENCES

1. R. H. Atalla, J. Appl. Polym. Sci., Appl. Polymer Symp. 28:659-69(1976).
2. R. H. Atalla, Interim Report, Project 3288, The Institute of Paper Chemistry, Sept. 28, 1979.
3. S. M. Ehrhardt, Doctor Dissertation, The Institute of Paper Chemistry, in press.

INSTRUMENTATION AND EXPERIMENTAL METHODS

R. Atalla and U. Agarwal

A substantial effort has gone into methods development and optimization of the Raman microprobe system. Two key improvements are described here. The first involved optimization of the Raman microprobe and development of more reliable procedures for analysis of polarization effects. The second was in the area of sample preparation and mounting.

INSTRUMENT OPTIMIZATION

Three different factors were integrated in optimization of the Raman microprobe system. The first was based on the realization that dichroism in the optics of the microprobe and the monochromator could confuse the analysis of spectrum variation as the polarization of incident light was altered relative to the geometry of morphological features. The decision was, therefore, made to keep the plane of incident polarization stationary with respect to the optical system. Rotation of polarization relative to morphological features was accomplished instead by installing a rotating stage on the microprobe, and rotating the samples about the optical axis.

The second modification grew out of the observation that the dichroism of the microprobe favored a polarization that is least efficiently transmitted by the monochromator. This problem was dealt with by installing a polarization scrambler along the optical axis of the microprobe. A significant increase in through-put was observed after installation of the scrambler.

The third factor was a procedural one for alignment of the overall system. The procedure used by the manufacturer to align the system involves mounting a

helium neon laser in place of the photomultiplier tube and aligning the components so as to maintain the beam along the optical axis of the system. In the belief that this procedure could allow minor departures in the alignment to escape undetected we sought a more precise procedure.

The method we developed was based on mounting a micrometer slide above a front surface mirror on the stage of the microprobe, illuminating with the laser exciting line, and following the image of the micrometer through the different optical components in the microprobe and the monochromator. At each stage the components were aligned to obtain a centered image. Upon completion of the process, an image of the micrometer slide could be observed in the plane of the photocathode of the photomultiplier tube. Upon conclusion of this procedure we observed an order of magnitude improvement in the quality of the signals.

SAMPLE PREPARATION AND MOUNTING

In the preliminary studies the samples sectioned to approximately 20 to 30 micron thickness were extracted and washed, then dried in air and mounted on slides. The Raman spectra recorded from such samples showed weak Raman peaks superimposed on a strong fluorescent background. The cell walls frequently decomposed at higher laser powers. In an attempt to obtain better quality spectra wet mounting of the samples was tested.

In the wet mounting procedure the samples were placed on a slide, covered with water, and sandwiched with a glass cover slip. A thin layer of silicone grease was deposited around the cover slip in order to stop water evaporation. In this procedure the sample could withstand higher power levels of the incident laser light and the background fluorescence was significantly reduced. In addition in some instances H_2O was replaced with D_2O as the immersion medium in order to avoid

overlap between the OH stretching bands and the CH stretching bands. In this procedure, however, it was found that the presence of the cover slips results in additional broad spectral features that could easily be assigned to the silicates in the glass. Use of Quartz cover slips, instead of the glass ones, improved the situation only marginally. The spectra of the cover slips might have been accounted for by subtraction, except that the relative intensities of the features varied from cover slip to cover slip, and sometimes from point to point within the same cover slip. This is not inconsistent with minor variation in the composition of the glass.

In order to avoid the contributions of the spectra of the cover slips, a new procedure was developed based on drilling small holes in the center of the cover slips. The wet sample was sandwiched as before, in between the cover slip and the slide; the cover slip would thus hold the sample immobile on the slide. The slide with the sample and cover slip was then inserted in a small beaker containing D_2O . The beaker was then mounted on the stage of the microprobe, and a water immersion objective, attached to the microprobe, lowered into the D_2O . In order to avoid evaporation of the D_2O a thin layer of mineral oil was placed on the surface of the D_2O , after the objective was entered into the medium. This procedure has resulted in the acquisition of the best spectra obtained so far from native woody tissue. Immersion in D_2O accomplishes a number of objectives. In addition to providing a heat sink to dissipate energy absorbed by the sample, the D_2O appears to quench the fluorescence of the native samples. Clearly it prevents dehydration of the samples, and it provides assurance that any spectral features observed in the OH stretching region are primarily associated with the woody tissue rather than absorbed H_2O .