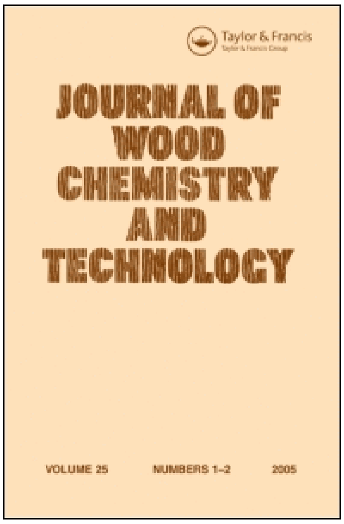

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RAMAN SPECTROSCOPY AND THE RAMAN MICROPROBE: VALUABLE NEW TOOLS FOR CHARACTERIZING WOOD AND WOOD PULP FIBERS

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ABSTRACT

A brief overview of Raman spectroscopy and the photon scattering phenomena which underly it is presented, together with the rationale for applying the methodology to the study of lignocellulosics. The sequence of studies undertaken at The Institute of Paper Chemistry to apply the methods to investigation of celluloses, chemical pulps, wood and high yield fibers are then reviewed.

The program began with studies on cellulose and related model compounds. These early efforts provided a basis for interpretation of the spectra of celluloses and for investigating the effects of various process variables on the aggregation of cellulose in pulp fibers. Among the effects touched upon are the differences between kraft and sulfite pulps, the effects of refining, and the influence of press drying on pulp crystallinity. More recently studies using the Raman microprobe made possible progress in the assignment of the vibrational spectra of cellulose and established the basis for using the microprobe to investigate variability of fibril orientation in native woody tissue.

In addition our extension of the studies to include lignin are noted. These included studies of fiber sections in native wood which show the orientation of lignin in the cell wall and observations of the variability of the ratio of lignin to cellulose.

Finally, the opportunities arising from new instrumental developments are reviewed. The new multichannel detectors will greatly enhance the efficiency of acquisition of spectra and allow

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Dedicated to Professor D. A. I. Goring in recognition of his valued contributions to physical chemical studies of wood and it components, and in appreciation of many stimulating discussions over the years.

more comprehensive explorations of the architecture of fibers. In addition, gated detectors coupled with pulsed laser excitation will allow studies of woody tissue and of high yield pulps that would not be feasible with single channel detection and continuous laser excitation.

INTRODUCTION

Raman scattering was first observed in 1928, and was used to investigate the vibrational states of many molecules in the 1930s. Spectroscopic methods based on the phenomenon have been used in research on the structure of relatively simple molecules since the early days. Over the past twenty years, however, the development of laser sources and new generations of monochromators and detectors have made possible application of Raman spectroscopy to the solution of many problems of technological interest.

In many industrial analytical laboratories Raman spectroscopy is routinely used together with infrared spectroscopy for acquisition of vibration spectra from materials under investigation. At least two instrument manufacturers are preparing to market Raman spectrometer systems in the $25,000 to $35,000 price range, which places them below the price of FTIR instruments. An important expansion of the potential of the technique has arisen from the use of the Raman microprobe, which permits acquisition of spectra from domains as small as 1 micrometer.

In 1970 the Institute initiated a program with the objective of understanding the states of aggregation of the natural polymers which constitute wood fibers and which determine their influence on properties. The approach is similar to that frequently applied to synthetic polymers within the framework of modern materials science. As part of this broader program an effort was undertaken to develop the application of Raman spectroscopy for characterizing the structure of fibers and in addressing problems arising in pulp and paper technology.

The program has progressed from studies of cellulose and related model compounds to studies of pulps and their response to different process conditions, and more recently to studies of lignins in wood and high yield pulps. This report provides an overview of the progress, the areas currently under active investigation, and the areas opened up by the new generation of systems for Raman spectroscopy.

In the next section primary phenomena and events in Raman scattering are discussed in relation to the parallel processes in infrared absorption. In addition, the section includes a review of the special advantages of Raman spectroscopy in investigating lignocellulosic systems. Next, the key questions in interpretation of the spectra are discussed together with the approach adopted in the Institute's program. Highlights of key results at each stage of the program are reviewed. Finally, the new areas of investigation opened up by the microprobe and by time resolved spectroscopy are outlined. Some results of microprobe studies using single channel detection are presented together with very preliminary results from multichannel systems.

RAMAN SPECTROSCOPY

The phenomena underlying Raman spectroscopy can be described by comparison with infrared spectroscopy as shown schematically in Fig. 1. There it is seen that the primary event in infrared absorption is a transition of a molecule from a ground state (M) to a vibrationally excited state (M^*) by absorption of an infrared photon with energy equal to the difference between the energies of the ground and excited states. The reverse process of infrared emission occurs when a molecule in the excited state (M^*) emits a photon during the transition to the ground state (M). In infrared spectroscopy, one derives information about a sample under investigation by measuring the frequencies of infrared photons it absorbs and interpreting these frequencies in terms of the characteristic vibrational motions of molecules known to absorb at these frequencies. In the case of more complex samples, some of the

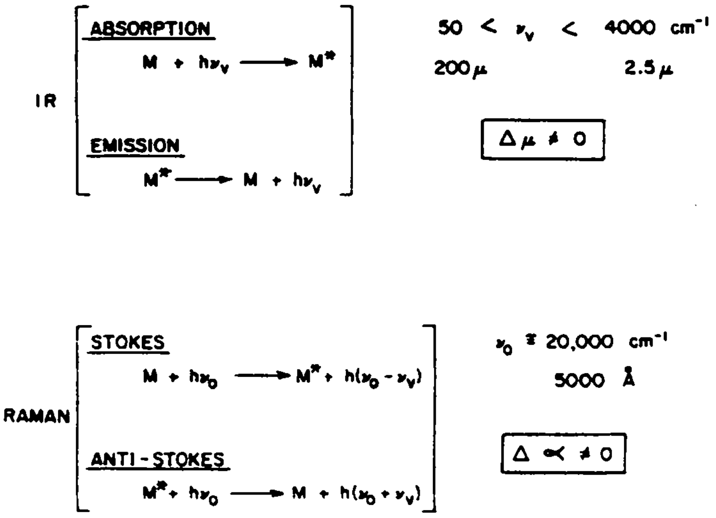

Fig. 1 Schematic representation of infrared and Raman processes. μ = Dipole movement, α = prolarizability, ν_v = vibrational frequency, ν_0 = exciting frequency.

frequencies are associated with functional groups that have characteristic localized modes of vibration.

As also shown in Fig. 1, the same transitions between molecular vibrational states (M) and (M^*) can result in Raman scattering. The key difference between the Raman and infrared processes are that the photons involved are not absorbed or emitted but rather shifted in frequency by an amount corresponding to the energy of the particular vibrational transition. In the Stokes process, which is the parallel of absorption, the scattered photons are shifted to lower frequencies as the molecules abstract energy from the exciting photons; in the anti-Stokes process, which is parallel to emission, the scattered photons are shifted to higher frequencies as they pick up the energy released by the molecules in the course of transitions to the ground state.

The exciting photons also are typically of much higher energies than those of the fundamental vibrations of most chemical bonds or systems of bonds, usually by a factor ranging from about 6 for OH and CH bonds to about 200 for bonds between very heavy atoms, as for example in I_2. In our work we most often use the 5145 Å line from Argon ion laser as the exciting frequency.

Measurement of a Raman spectrum requires exposure of the sample to a monochromatic source of exciting photons, and measurement of the frequencies of the scattered photons. Because the intensity of Raman scattering is much lower than that of Rayleigh scattering, a highly selective monochromator is required, and it must be coupled to a very sensitive detector. Rayleigh scattering, which occurs without change in frequency, arises from density variations and optical heterogenieties and is many orders of magnitude more intense than Raman scattering.

Figure 1 also depicts a key difference between Raman scattering and infrared processes. In order to be active in the infrared spectra, transitions must have a change in the molecular dipole associated with them. For Raman activity, in contrast, the change has to be in the polarizability of the molecule. These two molecular characteristics are qualitatively inversely related. For example, a molecule with a high dipole such as water has a low polarizability. As a consequence, though water absorbs very strongly in the infrared, its Raman scattering is quite weak, and it is almost the ideal solvent for Raman spectroscopy.

The difference between the conditions for activity in the infrared and Raman spectra makes the information from the two forms of spectroscopy complementary in most instances. Symmetric vibrations of highly covalent bond systems result in intense Raman bands and in weak infrared bands. On the other hand, asymmetric vibrations of highly polar bonds result in very strong infrared absorption and weak Raman bands. For example, while the strongest atmospheric bands in infrared absorption are the water bands and the asymetric bands of CO_2, the strongest atmospheric bands in Raman spectra are those of the homonuclear N_2 and O_2, which are transparent to the infrared.

STUDIES OF CELLULOSES AND PULP FIBERS

A number of considerations motivated the selection of Raman spectroscopy as a promising new tool for the study of lignocellulosic materials. The advances in infrared spectroscopy in the 1950s had led to a number of valuable studies of the spectra of cellulosic materials. Applications in the technology of cellulosics remained quite limited, however, because of two key difficulties. The first was the problem of optical heterogeniety and the resulting high degree of Rayleigh scattering of infrared photons by cellulosic materials. Because the degree of Rayleigh scattering depends on differences in the refractive indices at optical discontinuities and because the refractive index varies with wavelength in regions of strong absorption, it is difficult to separate extinction due to molecular absorption from extinction due to Rayleigh scattering.

The second problem confronting infrared absorption measurements arose from the hygroscopicity of cellulose. The high extinction due to water in a number of regions in the infrared spectrum made acquisition and interpretation of spectra difficult.

When laser sources and the new generations of monochromators and detectors became available for Raman spectroscopy, it seemed possible that its complementary relationship to infrared absorption might make the technique the better one for investigating the vibrational spectra of cellulosic materials. It became clear early in the assessment that the two primary difficulties in application of infrared spectroscopy will not arise with Raman measurements. The most intense bands in the spectra of cellulose would be associated with skeletal motions involving C-C and C-O bonds, with the hydroxyl groups and adsorbed water contributing only weakly. Furthermore, by the nature of the measurement process, Rayleigh scattered photons, which are at the excitation frequency are rejected by the monochromator. With these points in mind, a commitment was made to undertake to develop the methodology for acquisition and interpretation of Raman spectra of lignocellulosic materials.

The program envisioned involved a long term effort because the molecular systems of interest are complex, both chemically and vibrationally, and prior work provided very little basis for interpretation of spectra. The plan which guided the work was to begin with the most homogeneous chemical constituent of wood fibers, that is, cellulose. This would be followed by bleached chemical pulps, lignins, wood, and high yield pulps. At every stage, as progress was made in our understanding of the spectra, efforts would be made to use their measurement to characterize the effects of process steps.

The first objective thus was to investigate the Raman spectra of cellulose and the vibrational spectra of a number of sets of model compounds that would provide a basis for interpretation of the spectra of cellulose. The approach to investigating the spectra of cellulose was a semiempirical one based on perturbing the physical structure or state of aggregation of the cellulose and observing the resulting changes in the Raman spectra. The studies of model compounds were based on undertaking normal coordinate analyses of the vibrational motions of sets of related molecules and comparing these to the observed Raman and infrared spectral band of these compounds.

The studies of the model compounds led us to the finding that, with a few exceptions associated with highly localized vibrations involving hydrogen atoms, most of the modes of vibration were very highly coupled and delocalized. Thus the group frequency approach usually used in interpretation of infrared spectra is not applicable to the molecular chain modes of polysaccharides. Group frequencies are usually associated with highly localized modes which are characteristic of particular functional groups; the effect of coupling with adjacent bonds is often a relatively small shift in frequency. In retrospect, the failure of the group frequency method should not have surprised us, because pyranose rings and polymers thereof are made up of systems of C-C and C-O bonds. These bonds have similar reduced masses and bond energies, so their vibrational frequencies are close enough for a high degree of coupling to occur.

The studies of model compounds, though they raised questions about the group frequency approach, nevertheless provided valuable information about the different types of vibrational motions and the regions of the spectra within which they make their greatest contribution. This in turn allowed us to interpret the changes observed in the spectra of celluloses as they were subjected to structural perturbations.

Model Compound Studies

The groups of model compounds studied included the 1,5-anhydro pentitols,[1,2] the pentitols and erythritol,[3] the pentoses,[4] glucose,[5] the inositols,[6,7] and the cellodextrins.[8] In each instance a number of members of the group was used to develop a force field to describe the molecular vibrations, and the quality of the fit was tested by the ability of the force field to predict the vibrational modes of other members in the group which had not been used in development of the force field. Although there are occasional variations in detail, certain patterns emerge, and these should in most instances be equally valid for cellulose and other related polysaccharides.

The C-H and O-H stretching bands occur in the ranges of 2800 to 3000 cm^{-1} and 3000 to 3600 cm^{-1}, respectively, and are much removed from the frequencies of the other motions which are below 1500 cm^{-1}. The only localized mode below 1500 cm^{-1} is the HCH bending motion at C6 of the anhydroglucose residue; it usually occurs between 1440 and 1500 cm^{-1}. The bands between 1200 and 1440 cm^{-1} are due to modes involving considerable coupling of methine bending, methylene rocking and wagging, and COH in plane bending; these involve angle bending coordinates which include one bond to a hydrogen atom. Significant contributions from ring stretching begin below 1200 cm^{-1}, and these modes together with C-O stretching motions dominate between 950 and 1200 cm^{-1}. Below 950 cm^{-1}, angle bending coordinates involving heavy atoms only (i.e., CCC, COC, OCC) begin to contribute, though ring and C-O stretches and the external modes of the methylene group may be major components. The region between 400 and 700 cm^{-1} is dominated by the heavy atom bending, involving both the C-O bonds and ring motions, although some ring stretching modes still make minor contributions. In some instances, O-H out-of-plane motions may contribute in this region also. Between 300 and 400 cm^{-1} the ring torsions make some contribution, and below 300 cm^{-1} they generally dominate.

In addition to the above generalizations concerning modes which occur in one or another of the classes of model compounds investigated, the spectrum of cellulose can have components due to modes centered at the glycosidic linkages. Computations for cellobiose indicate that these modes are strongly coupled with modes involving similar coordinates in adjacent anhydroglucose rings.

Studies on Cellulose

The perturbations of structure that were the basis of our investigations of the Raman spectra of cellulose were mercerization and regeneration from solution; both were known to result in polymorphic changes and to have significant effects on properties. Our initial studies, which focused on mercerization, led us to the conclusion that celluloses I and II must correspond to different molecular conformations as well as different crystalline lattices.[9,10] Our studies of regeneration also supported these findings.[11]

In these early studies it was proposed that the two conformations represented small right-handed and left-handed departures, respectively, from the accepted twofold helix structures. In a subsequent study it was noted that adjacent anhydroglucose rings appeared nonequivalent,[12] and this was confirmed by Solid State ^{13}C NMR spectra.[13,14] This led to a redefinition of the two stable conformations in terms of two stable states of anhydrocellobiose as the repeat unit, with the glycosidic linkages alternating successively between right-handed and left-handed departures from the twofold helix conformation. This new model also permitted development of a method for quantitative analysis of the Raman spectra to establish the conformational distributions of samples of cellulose.[15]

More recently, the Raman microprobe has made possible adaptation of some of the methods used in studies of the spectra of single crystals to studies of aggregates of fibrils of cellulose. The fibrils are known to have the molecular chains aligned parallel to their axes. Thus, by recording spectra with the electric vector of the exciting laser beam at different angles to the fibril axes it is possible to identify the vibrational modes of the molecules according to their direction relative to the axes of the molecules. In addition to advancing the assignments of the modes of cellulose, these studies shed new light on the nature of the differences between the highly crystalline algal celluloses and those from the higher plants.[16,17]

Studies on Chemical Pulps

Development of the procedure for quantitative resolution of the spectra of cellulosic samples provided the basis for analyses of the spectra of samples of chemical pulps. Although differences between the spectra of celluloses from different plant sources had been noted, most could be interpreted in terms of differences in crystallinity or hemicellulose content or both.

In general, direct comparison of the spectra of pulps of commercial interest revealed few meaningful differences that could be associated with differences in process conditions. This was particularly the case if the samples had been derived from similar wood species by processes involving the higher temperatures associated with most commercial pulping processes.

An important step forward was associated with our recognition of the sensitivity of conformational change to mechanical restraint. While this was well known in connection with merdelignified to varying degrees. Spectra of the native lignin were then derived by subtraction. Interpretation of the spectra, however, had to await studies on model compounds.

A study of model compounds was undertaken; it was based on normal coordinate analyses of both infrared and Raman spectra.[23] The study included three monosubstituted, three disubstituted, and one trisubstituted model related to the common lignin fragments. Though the study provided a basis for assigning a number of features in the Raman spectra of the native lignins, the result most relevant in the present context was confirmation of the assignments of the aromatic ring stretching band at 1600 cm^{-1} and the alpha carbonyl band at about 1640 cm^{-1}.

The assignments enabled us to move on to one of the most exciting new developments in the area, that is, examination of molecular orientation of lignin in cell walls of native woody tissue. The Raman microprobe allowed acquisition of spectra from domains as small as one micrometer.[24,25] It became possible in that context to measure the effect of the polarization of the exciting laser radiation on the intensity of the symmetric aromatic ring stretching band at 1600 cm^{-1} while simultaneously analyzing the polarization of the Raman scattered photons. The results of such observations, illustrated in Fig. 2, can provide information concerning the orientation of the aromatic rings with respect to the electric vector of the incident beam and hence also indicate the orientation relative to the plane of the cell wall. The observations can also provide information about the relative amounts of lignin and cellulose at a particular point in the cell wall.

With the Raman microprobe it was possible for the first time to explore the degree of heterogeniety of structure in the cell walls. While in the first instance such information is important for understanding the molecular architecture of the cell wall, it has very important implications for the analysis of pulping processes, and studies of fiber properties, particularly in the case of high yield pulps.

cerization of textile fibers, its relevance to pulp characterization had not been immediately obvious. In two key studies in this area, we discovered that treatment of pulp samples with caustic solutions at the threshold of the mercerizing range could reveal differences in the degree of internal constraint on conversion from cellulose I to cellulose II.

In the first of these studies we detected a difference between kraft and sulfite pulps derived from birch.[18,19] We found that the kraft pulp was significantly less converted when treated with 11% NaOH solutions. We interpreted this observation in terms of constraints on the freedom of cellulose molecules to undergo conformational changes. It was speculated that cross links of chemical or mechanical origin were present to a greater extent in the kraft pulp and were responsible for this constraint.

We next examined the effect of mechanical refining on the degree of internal constraint within pulp fibers. It was found that moderate refining of some pulps had little effect on the susceptibility to conformational transformation. However, extensive refining was found to increase the susceptibility to transformation, suggesting that the process does indeed result in reduction of the constraints of morphology.[20,21]

In yet another application of Raman spectroscopy to process analysis, the Raman microprobe was used to investigate the effects of press drying on the structure of pulp fibers.[22] It was found that the higher heat fluxes associated with press drying result in enhancement of the rate of crystallization in pulp fibers. The crystallization, which was measurable by quantitative analysis of the Raman spectra, appeared to contribute to stiffening of fiber aggregates in press dried sheets.

Lignin and Wood

More recently we have expanded our effort to include the study of lignin in native wood, in groundwoods, and in high yield pulps. In the earliest work spectra were recorded from samples of wood

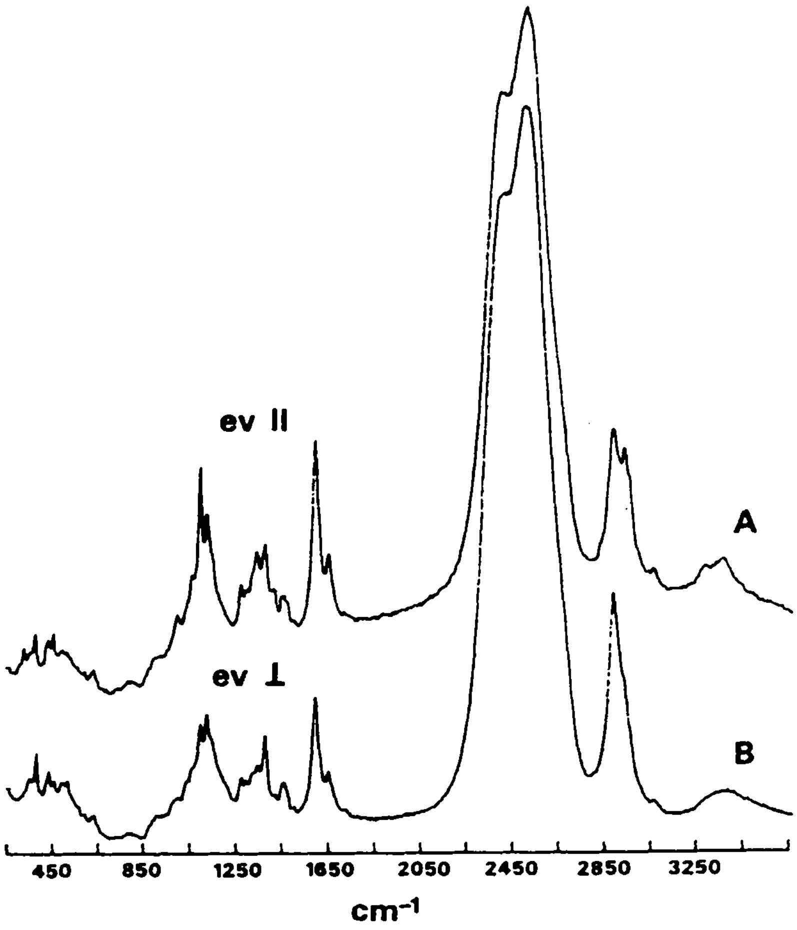

Fig. 2 Raman spectra from a domain approximately 1 μm in diameter in a longitudinal section of the secondary wall. (A) Electric vector parallel to the plane of the cell wall. (B) Electric vector perpendicular to cell wall.

The key findings, reported in greater detail elsewhere,[26,27] were that the aromatic rings of the phenylpropane units of lignin are preferentially oriented with respect to the plane of the cell wall. Most often the orientation is in the plane of the cell wall, but in some instances it is perpendicular, while in other instances there is no preferential orientation. Our conclusion has been that lignin is more highly organized than had heretofore been assumed.

Another set of findings concerned the compositional variation within cell walls. It was found that the ratio of lignin to cellulose varied across the cell walls and that the variability is greater between adjacent cells than between different points within the same cell.

Future Studies with Multichannel Detectors

In the work reported so far, the spectra have been acquired with a Raman system that relied on a single channel detection system. That is, the spectra are recorded by scanning the frequency range of interest and observing the intensity of Raman scattered light at each frequency in sequence. In such a system the spectra shown in Fig. 2 required multiple scans over a period of 6 to 8 hours to achieve acceptable signal/noise ratios. Acquisition of data from a number of different locations on the cell wall sufficient to allow statistical analysis would require a prohibitive amount of time, of the order of weeks for a single cell.

To overcome the difficulty we are now assembling a Raman microprobe system equipped with multichannel detection; the spectral interval of interest can be covered simultaneously by a diode array detector. We have demonstrated that spectra of signal/noise ratio equal to that in Fig. 2, can be acquired in 5 minutes, albeit with some limited sacrifice in spectral resolution.

In addition to facilitating more comprehensive mapping of the variability in cell wall architecture in different types of tissue, the new microprobe will make possible studies of diffusion of chemical reagents through the cell wall. Thus, it will provide data on mass transfer processes in cell walls and shed light on their role in determining key process rates in chemical pulping.

Another application of the Raman system with multichannel gated detection will be analysis of the evolution of excited states in lignin. This will be carried out using pulsed laser excitation, and should contribute to basic understanding of phenomena associated with color degradation in pulps, particularly high yield pulps.

In addition to the applications outlined above, gated multichannel detection and pulsed laser excitation are expected to permit application of Raman spectroscopy to address a number of problems in pulping and papermaking technology that could not be addressed with continuous laser excitation and single channel detection. We look forward to reporting on these in the future.

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