Chapter 9

An Overview of Raman Spectroscopy as Applied to Lignocellulosic Materials

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INTRODUCTION

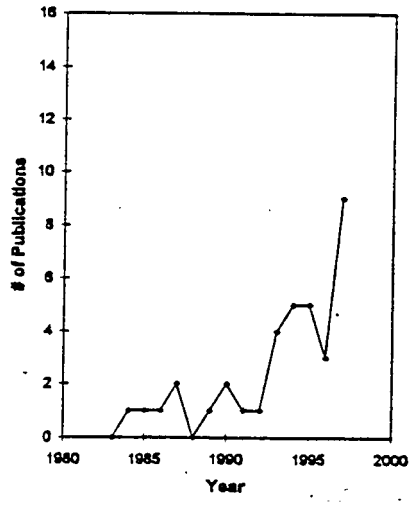
Compared with most materials, lignocellulosics have only been recently studied using Raman spectroscopy. Although Raman spectroscopy has been around for more than 60 years, compared with infrared (IR) spectroscopy its usage (even in other disciplines) has not been wide spread. This is because of a number of factors, including cost of Raman instrumentation, user unfamiliarity with the technique, and an impression that Raman provides information that is already available by IR. Actually, both Raman and IR spectroscopes complement each other.

For materials composed of cellulose, the situation started to change in the 1970s when results of Raman spectroscopic investigations began to appear in the literature [*1 - 4*]*;* lignin-contaiing materials were not studied until much later [*5, 6*]. However, with Raman studies of cellulosic samples came the awareness that most of these samples produced laser-induced fluorescence (LIF) [*2, 7*] –an intense background signal in a Raman spectrum that almost swamps the weaker Raman signal. Although a fact of work life for most Raman spectroscopists, the LIF contribution was highly undesirable because it not only deteriorated the quality of a Raman spectrum but, for certain samples, completely masked their Raman features. Later, while studying woody tissues and other lignocellulosics, the problem of LIF was encountered again and methods to deal with it had to be developed [*8, 9*].

As can be concluded from the foregoing comments, for quite some time there had been a need to solve the problem of LIF in Raman spectroscopy. For several years, Raman spectroscopists have recognized that one of the better ways to avoid LIF was to choose an excitation wavelength where a sample and its impurities do not absorb. For most samples, such a wavelength will lie in the near-IR region. Although as early as 1964 it was shown that a Raman spectrum can be obtained using a near-IR laser and an interferometer, it was not until 1986 that this achievement was put into practice [*10*]. Several advances in Raman instrumentation were needed to be in place before a practical system could be developed.

With the develpment of the near-IR excited Fourier-Tansform Raman (FT-) method, a renaissance occurred in the field of Raman spectroscopy. Numerous areas that were either previously not accessible to Raman spectroscopy or had suffered because of LIF saw renewed interest The field of lignocellulosics benefited as well [*11, 12*]. This can be noted in Figure 1 where "number of publications per year" data are shown for the past 16 years. It was not until 1984 that the first Raman spectrum of lignin and/or lignin-containing material was published [*5*]. Also, the number of yearly publications has been steadily increasing ever since FT-Raman instruments became commercially available.

Figure 1: Number of published papers (includes conference and journal papers but not meeting abstracts) compared with year of publication. When FT Raman instruments became commercially available, the number of publications steadily increased.



This review breifly covers both the fundamental and applied aspects of Raman spectroscopy in relationship to the field of lignocellulosics. The intent is to summarize the obtained information and make a potential user aware of the opportunities that Raman spectroscopy has to offer.

In the review, a significant amount of material presented is based on research that was carried out in the author's laboratory. This is by necessity because in the field of lignocelluiosics, other research laboratories have not yet used Raman spectroscopy extensively. However, wherever appropriate, published work from other laboratories

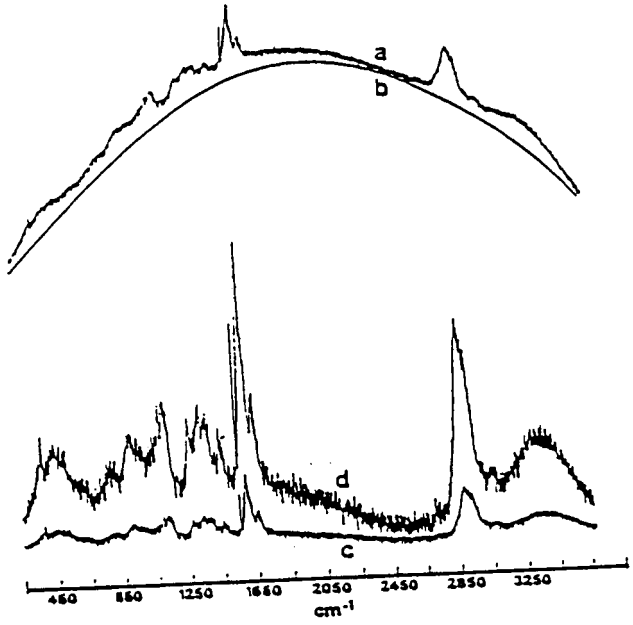
is included. It is hoped that many more laboratories will use Raman spectroscopy as a standard tool of analysis.

Conventional Compared With FT Raman

Prior to 1986, the only option was to use Raman instrumentation based on visible or UV excitation. Except for some highly specialized work, UV excitation was rarely used. (For lignocellulosics, compared with visible, UW excitation is likely to produce even higher fluorescence background because more lignin units fluoresce when excited at shorter wavelengths [*13*].) Most visible laser Raman systems were based on 488, 514.5 (both argon ion laser) and 647.1 (krypton ion laser) nm lines. Studies of lignocellulosics were most frequently carried out using the 514.5 nm line, although in one instance where the contribution of chromophores in pulps needed to be determined, 647.1 nm excitation was also used [*14*].

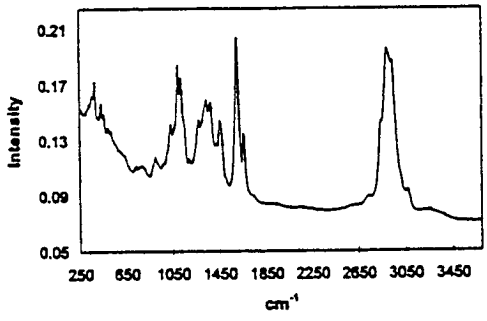
However, as commented previously, when lignocellulosics were studied using visible laser excitation LIF contributed strongly to Raman spectm The problem of LIF had been encountered before while studying synthetic polymers and other commercially produced materials [*15*]. In these cases, the cause of LIF was determined to be small amounts of residual impurities. It was discovered that better quality spectra could be obtained by allowing samples to remain in the laser beam for some time (drench quenching). Apparently, in drench quenching a laser beam photodegrades sample impurities. This method worked well for obtaining improved Raman spectra from cellulose samples [*3*]. However, in the case of lignin-containing materials, the method was not successful. The reason was that most of the LIF signal was from lignin itself. For such samples (except for ones that contain significantly modified lignin, *e.g.*, unbleached chemical pulps), the sampling under water and/or oxygen seems to work well [*8, 9*]. **A** Raman spectrum of dry black spruce section obtained under the conditions of oxygen flushing is shown in Figure 2. In contrast, water immersion sampling technique worked well for experiments carried out using a Raman microprobe. The reader is referred to a review of previous work [*16*] for detailed information on this topic.

Figure 2: 5145 nm excited Raman spectrum of black spruce, (a) in the atmosphere of 50 lb/in^2 molecular oxygen, (b) fluorescence bacisground created by combing two Gaussians, (c) result when (b) is subtracted from (a), and (d) expanded (c). Note that sigal-to-noise is poor in spectrum (d) due to LIF.



Although through use of a conventional Raman system (visible excitation) reasonably good quality spectra of most materials could be obtained (especially if a spectrum is obtained after averaging over a number of scans), there was a definite need to solve/avoid the problem of LIF. This need was met with the development of near-IR FT-Raman spectroscopy (sample excitation at 1064 nm or other near-IR wavelengths) [*10*]. In addition to avoiding the generation of LIF (for most samples), a spectrum could be obtained much more rapidly [*17*]; compared with several hours, a spectrum could be obtained in few minutes. For comparison purposes, an FT Raman spectrum of black spruce is shown in Figure 3.

Figure 3: Near-IR (1064 nm excited) FT Raman spectrum of spruce wood; signal-to-noise is much improved compared with spectrum shown in Figure 2.



In addition to the difference in laser wavelengths involved (visible in conventional versus near-IR in FT Raman), the manner in which the two techniques process Raman signal is different [*17*]. An FT spectrometer uses a Michelson interferometer instead of dispersive gratings (in conventional Raman to analyze the scattered light. The overall throughput of an FT Raman system is much improved even when the loss in scattering efficiency due to significantly longer wavelengths of Raman lines (compared with visible Raman) is taken into consideration. This is the reason why an FT Raman instrument takes much less time to record a spectrum. An additional advantage associated with the FT approach is that, in a spectrum wavenumber values are more accurate (compared with visible Raman); therefore, the results of spectral subtraction are likely to be better.

Macro Compared With Micro Raman

For *in situ* structural analysis of lignocellulosic materials, which are heterogeneous composites of cellulose, lignin, and hemicellulose and whose microstructures are composed of morphologically distinct regions, Raman spectroscopy is a good technique. Capability to analyze microscopic regions using a microprobe is another important tool in the arsenal of Raman spectroscopy [*18*]. Further considering that presence of water in a sample is not a problem (unlike IR) and information on the orientation of macromolecular components can be obtained Raman spectroscopy has capabilities that are not provided by any other method. Therefore, Raman is capable of providing unique information when macro- and micro-investigations of materials are carried out.

If samples are to be analyzed at the microscopic level, a microprobe–either conventional or FT Raman be used, although spatial resolution is much better in the former case. For a 514.5 nm excitation based Raman microprobe, a spatial resolution of 1.6 micrometer (μm) was obtained using a 100x microscopic objective. In contrast, using the same magnification objective, an FT system provided a resolution of about 10 μm. Moreover, in an FT microprobe, the sampling depth is significantly larger. To study heterogeneous samples like woody tissues, where distinct morphological regions need to be analyzed at highest possible spatial resolution, a conventional microprobe is more useful. Nevertheless, if information from 10 μm or larger sample regions is required, an FT Raman microprobe should be used.

Band Assignment

Assignment of bands in the Raman spectra of lignocellulosics is an important topic of research Although some information is already available [*19, 20*], research in this area needs to be accelerated considering that more and more lignin-containing materials are being studied using Raman spectroscopy. For interpreting the Raman spectrum of a multi-component material like lignocellulose, not only the contribution of each component needs to be identified but the latter needs to be assigned to component-specific structural units and/or functional groups. For example, this approach has been adapted for assigning Raman spectrum of black spruce [*21*]. Although this work is not yet complete, most significant spectral features (in the Raman spectrum of black spruce) have now been assigned at both component and functional group levels.

In this context, note that Raman features of cellulose have already been assigned [*22*]. Moreover, hemicellulose spectral assignments are expected to be very similar to that of cellulose [*21*]. Therefore, it is primarily lignin for which bands need to be assigned. Assignment for softwood-cellulose Raman bands is given in Table 1.

Table 1: Assignment of bands in the FT-Raman spectrum of softwood-cellulose

Band (cm^{-1})	Assignment[a]
330 sh[b]	heavy atom bending
351 w	some heavy atom stretching
380 m	some heavy atom stretching
406 vw	?
435 m	some heavy atom stretching
458 m	some heavy atom stretching
492 w	?
520 m	some heavy atom stretching
899 m	HCC and HCO bending at C6[c]
971 vw	heavy atom (CC and CO) stretching
1000 vw	heavy atom (CC and CO) stretching
1037 sh	heavy atom (CC and CO) stretching
1063 sh	heavy atom (CC and CO) stretching
1073 sh	heavy atom (CC and CO) stretching
1095 s	heavy atom (CC and CO) stretching
1123 s	heavy atom (CC and CO) stretching
1149 sh	heavy atom (CC and CO) stretching plus HCC and HCO bending
1298 sh	HCC and HCO bending
1338 m	HCC and HCO bending
1377 m	HCC, HCO, and HOC bending
1456 m	HCH and HOC bending
2740 vw	?
2848 sh	CH and CH_2 stretching[d]
2895 vs	CH and CH_2 stretching

[a]Assignment based on reference [22].
[b]Note: vs is very strong; s is strong; m is medium; w is weak; vw is very weak; sh is shoulder. Band intensities are relative to other peaks in the spectrum.
[c]In reference [22] the band is at 913 cm^{-1}.
[d]In reference [22] the band is at 2868 cm^{-1}.

Further considering that lignin and hemicellulose molecular structures are somewhat different in different lignocellulosic materials *(e.g.*, in softwood, hardwood, and grasses), it is even more important that the goal of band assignment for each class of differing lignocellulosics be accomplished. When assignments of bands are available,

one can evaluate how structural differences and similarities of lignin and carbohydrate polymers are reflected in their individual Raman spectra. For black spruce (softwood) lignin, Raman bands have been assigned [*19*]. They are reproduced

Table 2: Assignment of bands in the FT-Raman spectrum of softwood lignin

Band (cm^{-1})	Assignment[a]
357 w[b]	skeletal deformation of aromatic rings, substituent groups and side chains
384 w	skeletal deformation of aromatic rings, substituent groups and side chains
463 vw	skeletal deformation of aromatic rings, substituent groups and side chains
491 vw	skeletal deformation of aromatic rings, substituent groups and side chains
537 vw	skeletal deformation of aromatic rings, substituent groups and side chains
555 vw	skeletal deformation of aromatic rings, substituent groups and side chains
591 vw	skeletal deformation of aromatic rings, substituent groups and side chains
634 vw	skeletal deformation of aromatic rings, substituent groups and side chains
731 w	skeletal deformation of aromatic rings, substituent groups and side chains
787 w	skeletal deformation of aromatic rings, substituent groups and side chains
900 vw	skeletal deformation of aromatic rings, substituent groups and side chains
926 vw	CCH wag
969 vw	CCH and -HC=CH- deformation
1033 w	C-O of aryl-O-CH_3 and aryl-OH
1102 w	out of phase C-C-O stretch of phenol
1134 m	a mode of coniferaldehyde
1191 w	a phenol mode
1216 vw	aryl-O of aryl-OH and aryl-O-CH_3; guaiacyl ring (with C=O group) mode
1271 m	aryl-O of aryl-OH and aryl-O-CH_3; guaiacyl ring (with C=O group) mode
1297 sh	aryl-O of aryl-OH and aryl-O-CH_3; C=C stretch of coniferyl alcohol
1333 m	aliphatic O-H bend
1363 sh	C-H bend in R_3C-H
1393 sh	phenolic O-H bend
1428 w	O-CH_3 deformation; CH_2 scissoring; guaiacyl ring vibration
1454 m	O-CH_3 deformation; CH_2 scissoring; guaiacyl ring vibration
1508 vw	aryl ring stretching, asymmetric
1602 vs	aryl ring stretching, symmetric
1620 h	ring conjugated C=C stretch of coniferaldehyde
1658 s	ring conj. C=C stretch of coniferyl alcohol; C=O stretch of coniferaldehyde
2843 m	C-H stretch in OCH_3, symmetric
2886 sh	C-H stretch in R_3C-H
2938 m	C-H stretch in OCH_3, asymmetric
3007 sh	C-H stretch in OCH_3, asymmetric
3065 m	aromatic C-H stretch

[a]Assignment taken from reference [*19*].
[b]See footnote to Table 1.

Lignin Models

Lignin models have been studied in the context of band assignment work [*20, 23, 24*]. In the author's laboratory, more than 70 lignin models, representing various structural units of lignin, have been studied. In addition, a study was undertaken to understand how the band intensities of the benzene ring mode (at 1600 cm^{-1}) and of certain other functional groups (C=C and C=O) depended upon structural aspects of a model *(e.g.,* ring conjugation) [*25*]. These results provided insights into understanding why certain lignin structural units could be easily detected in Raman spectroscopy. The results were largely interpreted in terms of dependence of the scattering coefficients on pre-resonance Raman and conjugation effects-the two intensity enhancing mechanisms in Raman spectroscopy (see Pre-Resonance Raman and Conjugation Effects).

Another area where models are being used is in understanding how molecular environment affects spectral features. Depending upon the nature of intermolecular interactions (environment), a Raman peak could be shifted significantly and/or a band profile could be modified. Such information is useful in interpreting spectra of lignocellulosics. Solvation behavior of coniferaldehyde, a lignin model, was investigated using Raman and IR spectroscopes [*26*]. Coniferaldehyde was selected because in lignocellulosics it is expected to interact with the hydroxyl groups (of cellulose and hemicellulose) *via* hydrogen bonding. The results of solvation experiments indicated that phenolic hydroxyl and γ - keto groups of coniferaldehyde interacted strongly with molecules of various solvents. This supported the likelihood that coniferaldehyde interacts with hydroxyl groups in lignocelluiosics.

Pre-Resonance Raman and Conjugation Effects

For a given molecule when the laser excitation wavelength is chosen such that it coincides with an allowed electronic transition, the process of Raman scattering is called resonance Raman scattering [*27*]. This results in significantly enhanced intensities of certain Raman bands of that molecule. However, when the wavelength of excitation is such that it is significantly removed from the resonance condition, but the molecule is still able to absorb, the process is called pre-resonance Raman scattering. The band intensities in pre-resonance Raman are enhanced to varying degrees depending upon how far away from resonance the excitation wavelength lies. As the wavelength of excitation approaches pre-resonance and/or resonance, significant changes in intensity and frequency of Raman bands can occur. A detailed discussion of these topics is beyond the scope of this review, see reference [*27*] for additional information.

Considering excitation at visible wavelengths, only a few of the substructures of lignin are capable of exhibiting pre-resonance effect. None of the carbohydrate polymers are electronically excited. For black spruce lignin, it has been shown that using 514.5 nm excitation as much as 37% of the 1595 cm^{-1} band (in visible Raman) intensity is due to pre-resonance Raman effect [*28*]. Because of such a higher disproportionate contribution from a much smaller number of aromatic units, the 1595 cm^{-1} band could not be used for quantifying lignin in woods [*29*]. In contrast, this effect was used advantageously while identifying contributions of chomophores in unbleached and bleached thermomechanical pulps [*14*]. When spectra of these pulps were obtained using 514.5 and 647 nm excitations, Raman contributions of chromophores to specific bands were quantified. The results indicated that bleaching failed to completely remove chromophores in pulp.

It is known that the intensity of certain Raman vibrations is influenced by the degree of n-electron conjugation between aromatic ring and substituents [*30*]. This has implications for Raman scattering of lignin. This effect is known to be independent of wavelength of excitation. Because of this independence on wavelength, the conjugation effect can be easily distinguished from the pre-resonance Raman effect by exciting at a wavelength where the sample does not absorb.

Depending upon the wavelength of excitation and molecular structure, a Raman spectrum can have contributions from either pre-resonance Raman or conjugation effect. It is also possible that both these effects are present. Presence of such effects explains why contributions of some lignin units can be more easily detected than that of others. To investigate the dependence of scattering on molecular structure in lignin substructures, several model compounds (both conjugated and non-conjugated) were selected (Figure 4). Selected models, shown in Figure 4, are identified in Table 3.

Figure 4: Molecular structures of selected lignin models. Models are identified in Table 3.

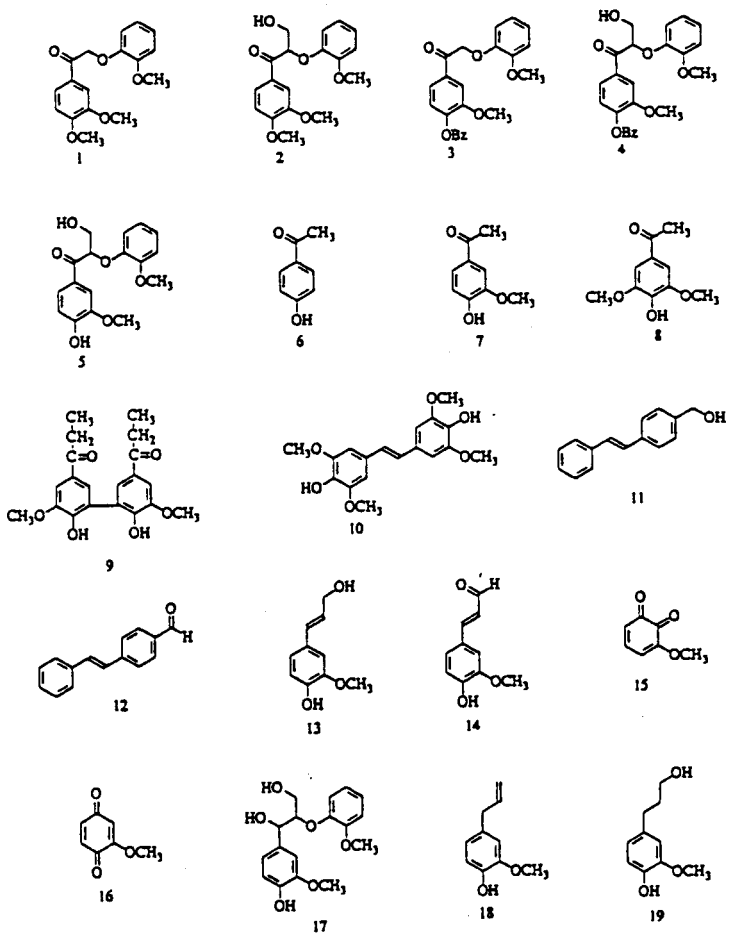
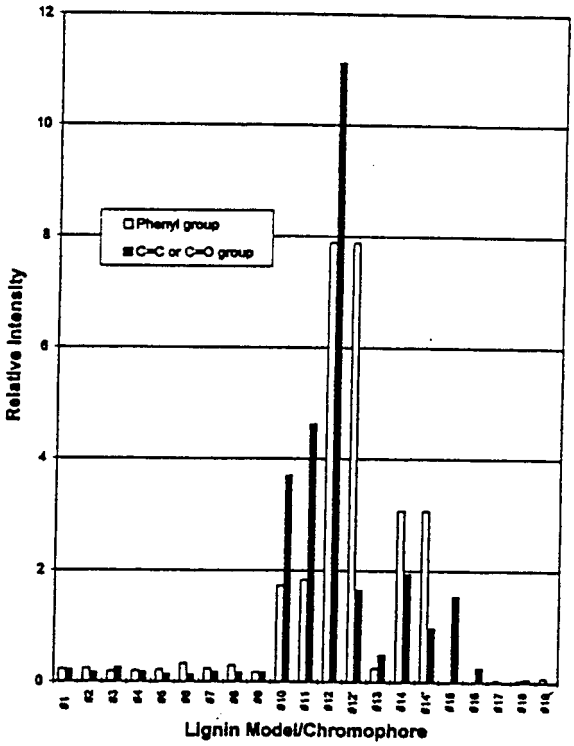


Table 3. Identification of model compounds shown in Figure 4

Model	Model name
1	1-(3,4-dimethoxy phenyl)-2-(2-methoxy phenoxy) ethanone
2	1-(3,4-dimethoxy phenyl)-3-hydroxy-2-(2-methoxy phenoxy) propan-1-one
3	1-(3-methoxy-4-benzoxy phenyl)-2-(2-methoxy phenoxy) ethanone
4	1-(3-methoxy-4-benzoxy phenyl)-3-hydroxy-2-(2-methoxy phenoxy) propan-1-one
5	1-(3-methoxy-4-hydroxy phenyl)-3-hydroxy-2-(2-methoxy phenoxy)-propan-1-one
6	4-hydroxy acetophenone
7	4-hydroxy-3-methoxy acetophenone
8	3,5-dimethoxy-4-hydroxy acetophenone
9	5,5'-bi-(4-hydroxy-3-methoxy propiophenone)
10	syringyl stilbene
11	stilbene methanol
12	stilbene carboxaldehyde
13	coniferyl alcohol
14	coniferaldehyde
15	3-methoxy o-quinone
16	3-methoxy p-quinone
17	1-(3-methoxy-4-hydroxy phenyl)-2-(2-methoxy phenoxy) propane-1, 3-diol
18	4-allyl-2-methoxy phenol
19	1-(3-methoxy-4-hydroxy phenyl)-propan-3-ol

These compounds were studied using a 514.5 nm laser-excitation-based Raman spectrometer. Relative Raman intensities with reference to the solvent (dichioromethane) band at 1420 cm^{-1} were calculated for the phenyl and C=C and/or C=O vibrations and are shown as a bar chart (Figure 5). Note in Figure 5 that highly conjugated structures are most easy to detect in Raman spectroscopy. In the visible and/or near-IR FT Raman spectroscopy, cellulose and hemicellulose do not show conjugation effect.

Figure 5: Relative Raman intensities (in 514.5 nm excited spectra) of lignin model compounds (for phenyl, C=O, and C=C groups). Key see Figure 4 and Table 3. For models 12 and 14, which contain both C=C and C=O groups, #12' and #14' solid bars represent intensities of C=O stretch mode.

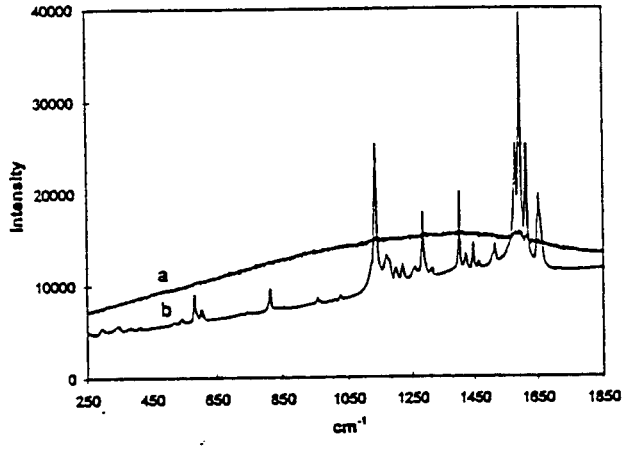


Surface Enhancement

Surface enhancement effect in Raman spectroscopy was first observed in 1973 [*31*]. In this effect, the intensity of Raman signal from a collection of molecules, that are adsorbed on a metal surface, is significantly enhanced (up to a million times). The surface enhancement depends upon the metal surface; silver, copper, and gold surfaces show the most enhancement. Therefore, if molecular interactions with the metal surface are such that they produce surface enhancement effect, trace amounts of a compound can be detected. However, not all chemicals would exhibit this phenomenon.

We studied certain lignin models to investigate whether such molecules could be made to display a surface enhancement effect. Although the effort is ongoing, so far only coniferaldehyde has shown the enhancement (Figure 6). In Figure 6, for a given concentration, compared with the surface enhanced spectrum, normal spectrum has bands that are hardly detected. If surface enhancement effect could be introduced for specific lignin structures, potential exists for this method to be used for detecting trace amounts of lignin substructures.

Figure 6: Normal (a) and surface enhanced (b) Raman spectra of coniferaldehyde. Note that bands in the normal spectrum are so weak that they are almost undetected.



Self-Absorption

In the context of Raman spectroscopy, self-absorption can be defined as the absorption of Raman scattered light by the sample itself. When this happens, the detector registers a lower signal due to the missing photons. In a spectrum this will be manifested through lowering of Raman intensity at the wavenumber position where the sample absorbs. This phenomenon has been mostly encountered in FT Raman spectroscopy [*32*] because absolute frequencies associated with Raman shifts lie in the near-IR spectrum region where overtone and combination bands of highly polar groups are detected. For example, water, if present in samples, can give rise to self-absorption.

The phenomenon of self-absorption in lignin, cellulose, and thermomechanical pulps has been studied in the author's laboratory. The results indicated that only the intensity of bands in the 2900 cm^{-1} region was impacted by this phenomenon. For thermomechanical pulps, the spectra (Figure 7) indicated that the intensity of the 2895 cm^{-1} band (relative to 1095 cm^{-1} band) depended on the position of the sample in the sampling comparment (Figures 7 and 8, distance in millimeter is sample position from the laser focus). In addition, when self-absorption was present, sample thickness also played a part by lowering the 2895 cm^{-1} band intensity. Furthermore, when water was deliberately introduced into a pellet of thermomechanical pulp, the 2895 cm^{-1} band declined significantly. The latter indicated that water in the sample is likely to be responsible for lowering most of the band intensity, although hydroxyls in cellulose and hemicellulose could also be responsible to a degree. These observations imply that if bands in the 2900 cm^{-1} region are to be used for quantification purposes (in FT Raman), it should be ensured that their intensities are not affected by self-absorption.

Figure 7: Effect of self-absorption on the intensity of the 2895 cm^{-1} band. Different spectra represent various positions of a thermomechanical pulp sample in the sample compartment; 1 mm means the sample is 1 mm away from the focus position.

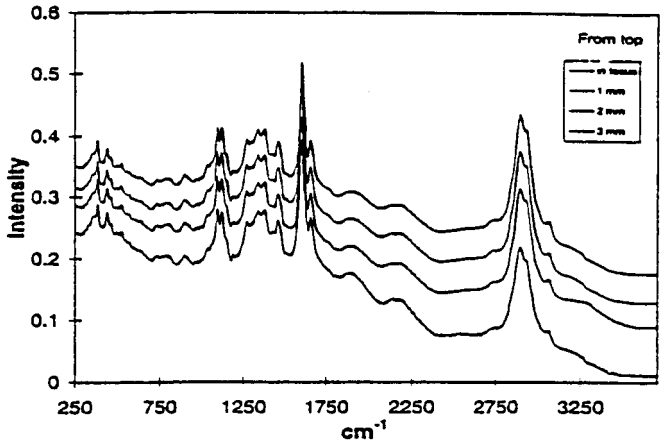
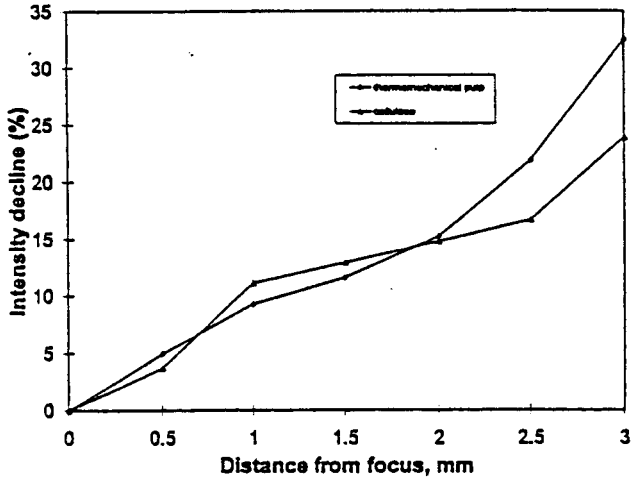


Figure 8: Decline in the 2895 cm^{-1} band intensity for thermomechanical pulp and Whatman #1 cellulose paper. Distance "0" corresponds to sample being in laser-focus position.



APPLICATIONS

Woods

Raman spectroscopy has been applied to study hadwoods and softwoods. Using FT Raman, spectral features of the two classes of woods have been compared and attempts were made to see if the technique could be used to differentiate these classes of woods **[***11, 12, 33*]. In one study [*33*] using 71 woods, neural computing method was applied to extract key spectral features were different between these species.

In other studies, attempts were made to assign bands in the spectra of wood [*20, 21*]. For instance, it was found in black spruce [*21*] that most of spruce Raman features could be assigned to cellulose and lignin. Contribution of hemicellulose occurred at wavenumber positions where cellulose contributed and was not easily detected. In another application, FT Raman in conjunction with partial least squares (PLS) regression method was used to determine constituents of eucalyptus wood [*34*].

Some studies of woody-tissue structure and organization have been carried out using a conventional Raman microprobe [*6, 28, 35, 36*]. One important result obtained was the evidence that lignin in the secondary walls of black spruce is oriented. Although it is not clear what caused this orientation, these results highlight the fact that Raman spectroscopy provides information that is unique. Addtional detailed cell wall mapping work is continuing. As another example of the usefulness of this technique, a study of comer middle lamellae in white birch and black spruce indicated that the concentration of lignin was not constant and could vary by as much as 100% [*36*]. This finding has important implications for understanding the ultrastructure of wood.

Another lignocellulose, cells of Zinnia elegans, has been analyzed for presence of cellulose, lignin, and how presence of cellulose inhibitor affects biosynthesis of lignin [*37*]. Raman information suggested that the presence of the inhibitor 2,6-dichlorobenzonitrile has an effect on lignin formation.

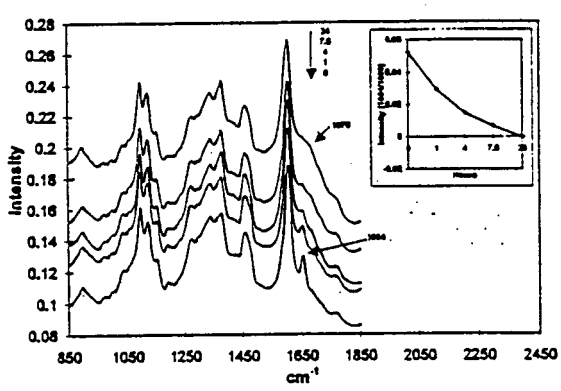
Adhesives and resins in wood have been detected *in situ* using FT Raman spectroscopy [*38, 39*]. Raman features, associated with these chemicals, have been used to study the process of resin penetration and curing.

Mechnical Pulps

Photochemistry

Photochemistry of mechanical pulps is an area that has benefited significantly from the use of Raman spectroscopy because, early on, this technique was applied to analyze light-induced changes [*24, 40*]. A time-resolved Raman analysis of thermomechanical pulps (Figure 9) clearly showed that exposure to light was responsible for the decay of the 1654 cm^{-1} band and appearance of a new feature at 1675 cm^{-1} [*40*]. The former band is known to have contributions from both coniferyl alcohol and coniferaldehyde groups [*41, 42*]. (Coniferaldehyde is present only in unbleached mechanical pulp.) And the band at 1675 cm^{-1} is due to *p*-quinones [*43*]. Therefore, using Raman it became clear that photoexposure caused the decay of coniferyl alcohol and coniferaldehyde units in lignin and was also responsible for formation of *p*-quinones groups. It is noteworthy that prior to FT Raman spectroscopy detection of *p*-quinones in photoexposed mechanical pulps has not been possible.

Figure 9. Time-resolved FT Raman spectra of photoexposed borohydride bleached thermomechanical pulp. The band at 1654 cm^{-1} declined with exposure (1 to 24 h) and a new feature at about 1675 cm^{-1} appeared as a result of chromophore formation. Inset shows decline of the 1654 cm^{-1}.



Bleaching

When mechanical pulps are bleached, lignin is not modified significantly and only chromophores in lignin are modified/removed. Depending upon the reductive *(e.g.,* borohydride) or oxidative *(e.g.,* hydrogen peroxide) nature of the bleaching agents, different reactions are expected to take place. Although useful insights have been obtained with the help of lignin models, accuracy of such information cannot be

evaluated because only limited amount of information has bee obtained from pulps. Raman spectroscopy has the potential to provide such additional information.

Both conventional and FT Raman spectroscopes have been used to study bleaching-related changes in mechanical pulps [*14, 23, 24, 40*]. It was demonstrated that bleaching with peroxide and borohydride removed coniferaldehyde groups. In addition, from the Raman spectra evidence indicating that p - quinones were modified and/or removed was obtained. The removed Raman contribution of *p*-quinones was found to be linearly correlated with the increase in pulp brightness (author's unpublished results). Thus, Raman spectroscopy provided evidence in support of the possibility that most mechanical pulp-brightening is due to removal/mmodification of *p*-quinone groups.

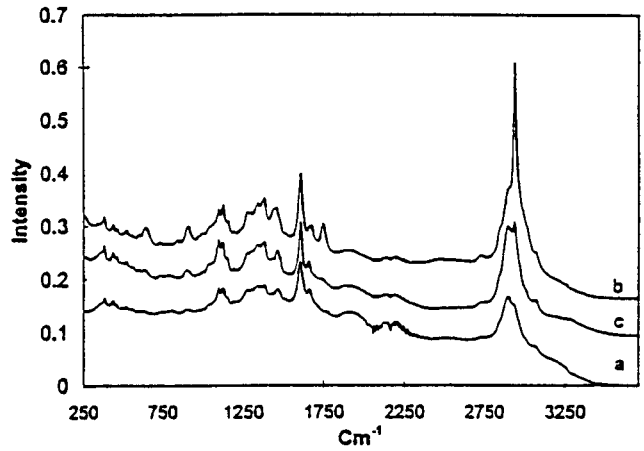
Chemical Reactions

Raman spectroscopy is being increasingly used to monitor chemical reactions [*44*]. In chemical industry, on-line monitoring capability is being developed using fiber-optic based Raman systems. In our laboratory, hydrogenation and acetylation of lignin and lignin-containing materials have been studied.

In the area of photoyellowing, there was a need to determine whether aromatic-ring conjugated C=C groups in coniferyl alcohol were completely hydrogenated in mechanical pulps. Using Raman, hydrogenation reaction was followed by monitoring the intensity decline at 1654 cm^{-1}. It was found that diimide, under modified reaction conditions, was able to completely hydrogenate a pulp [*40*].

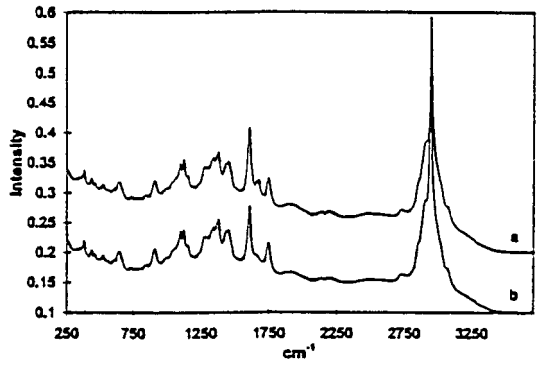
Acetylation and de-acetylation reactions of a mechanical pulp were also monitored using Raman. As shown in Figure 10, upon acetylation, most spectral change occurred at 2938 cm^{-1} as a result of incorporation of the acetyl groups in the pulp. Acetylation was considered to have been complete when, upon additional acetylation, the intensity of this band (relative to 1095 cm^{-1} band) did not increase further. Analogously, success of de-acetylation (of an acetylated sample) was evaluated by the decline of intensity at 2938 cm^{-1} (Figure 10c).

Figure 10: Raman spectra of control (a), acetylated (b), and de-acetylated (c) thermomechanical pulps. Upon acetylation, the most intensity enhancement is seen at 2938 cm^{-1} which can be used to monitor the completeness of the acetylation and/or de-acetylation reactions.



An analysis of photoexposed acetylated thermomechanical pulp was carried out to determine if its photoexposure caused any de-acetylation. A comparison of Raman spectra of control and light exposed samples indicated that no significant de-acetylation occurred (Figure 11). Intensity decline at certain band positions was due to degradation of coniferaldehyde and coniferyl alcohol structures.

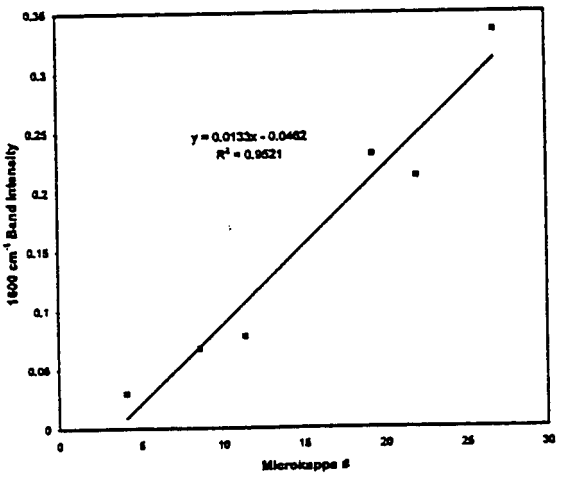
Figure 11: Acetylated pulp Raman spectra: (a) before photoexposure and (b) after 24 h photoexposure. Similarity of band intensity at 2938 cm^{-1} suggested that photoexposure did not cause any detectable de-acetylation in pulp.



Lignin Quanification

Several papers have been published where the Rama method has been applied for quantifying lignin in kraft pulps. In author's laboratory, the method was used to determine lignin amount in partially bleached kraft pulps using a variety of bleaching agents [*45*, *46*]. Although for a given bleaching sequence, good linear correlation between lignin's Raman band and kappa number could be obtained (see Figure 12), when pulps that were bleached using different sequences were analyzed together, the correlation between Raman band intensity (1600 cm^{-1}) and kappa was not as good. Additional studies are needed to improve upon the lignin quantification capability of Raman. Topics such as multivariate analysis *(e.g.*, Principal Component Regression, PLS), effect of mixed wood-species pulps on lignin band intensity, and the role of bleaching chemistry need additional investigation before a reliable model to predict lignin content can be developed.

Figure 12: 1600 cm^{-1} band intensity compared with kappa number for a series of CEDED. The linear correlation between Raman intensity and kappa number indicates that Raman spectroscopy can be developed to measure lignin content of bleached kraft pulps.



In addition to using Raman to quantify lignin in partially bleached pulps, where spectra are affected by bleaching chemistry, attempts were made to determine if the technique can be used to quantify lignin in softwood [*47*] and hardwood [*48*] kraft pulps. Raman spectra of pulps are likely to have variations due to differences in kraft pulping and differences in woods (within the class of softwood or hardwood). These

studies concluded that using Raman, lignin could be quantified in both softwood and hardwood kraft pulps. However, in this author's opinion, because two different calibration equations (for the two cases) were obtained the method is likely to be less reliable for analyzing samples that have both softwood and hardwood pulps.

Papers

Coated and uncoated papers have been studied using Raman spectroscopy. Spectra of coating mixture, latex, $CaCO_3$, and other components were used to analyze these papers [*49*]. Raman features of coated papers were interpreted in terms of these components and it was found that latex and $CaCO_3$ contributed strongly.

FT Raman spectroscopy was used to differentiate between sulfate and sulfite wood papers [*50*]. The study indicated that in addition to differences because of hardwood and softwood, sulfite paper spectra showed a band at 510 cm^{-1}.

Raman microscopy has also been used to analyze paper surface [*51*]. Researchers used this technique to study mottling (alternate light and dark printed areas). The role of latex concentration and distribution in mottling was studied, and it was concluded that this phenomenon was caused by the heterogeneity of the coating thickness and not by the heterogeneity of the latex distribution at the paper surface.

Other Lignocellulosics

In addition to wood pulp, and paper, Raman spectra of several other lignin-containing materials have been obtained. These include bamboo [*20*], kenaf [*52*], jute [*52*], corn, wheat sugarcane baggase [*52*], and flax [*53*]. Several of these materials were studied in the author's laboratory, and in most cases, good quality spectra were obtained.

In the spectrum of bamboo [*20*], strong lignin features were detected at 1604 and 1630 cm^{-1}. The bands were assigned to free and esterified phenolic units in lignin.

Studies of flax and its parts indicated that major components of each could be detected using Raman spectroscopy [*53*]. Bands of cellulose were the most intense features in the spectra Contributions of hemicelluloses were present in bast fibers and fibers. Signals due to pectins were weak and were detected in the bast cuticle/epidermis, fibers, and stem.

CONCLUDING REMARKS

For one reason or another, Raman spectroscopy has taken a lengthy time to fully develop. Now that the technique has reached this status, its application to the field of lignocellulosic needs to be explored vigorously. In some cases, this will be straightforward, whereas in other situations, the technique may require adaptation. In any case, the future for obtaining important information using Raman spectroscopy looks bright.

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