Agarwal, U. P.; Atalla, R. H. 2000. Using Raman spectroscopy to identify chromophores in lignin-lignocellulosics. ACS symposium series 742. Lignin: historical, biological, and materials perspectives. Washington, DC: American Chemical Society: Chapter 11. 250-264.

Chapter 11

Using Raman Spectroscopy to Identify Chromophores in Lignin-Lignocellulosics

U. P. Agarwal and R. H. Atalla

U.S. Department of Agriculture FS, Forest Products Laboratory, Madison, WI- 53705

Raman spectroscopy is being increasingly applied to study lignin and lignin-containing materials. One of the strengths of Raman is that it is highly sensitive to chromophoric structures. Various color-generating species are present in small quantities and are not easily isolated; therefore, the method is ideally suited to the study of chromophores *in* situ. Chromophore contributions can be easily detected because, except for the 1600 cm^{-1} band, which is due to lignin, the region where chromophores most strongly contribute (1500-1750 cm^{-1} region) is devoid of strong contributions from lignocellulosic components. Several chromophore lignin-models including quinones and stilbenes were studied and their Raman contributions were identified. This knowledge was then applied in the studies of photoyellowed thermomechanical pulp (TMP), bleached TMP, and acid-hydrolyzed TMP. In all cases, Raman studies provided important information on presence or absence of various chromophore groups in pulps.

The development of near-IR FT Raman spectroscopy has made it possible to acquire good quality spectra of most lignocellulosic materials [*1*]. Moreover, with the availability of commercial instruments Raman analysis of such materials has become routine [*2*]. In our laboratory, Raman spectroscopy has been used to obtain important information on a number of lignocellulosic materials [*3-6*]. Considering that Raman spectroscopy is complementary to IR, both techniques should be used to obtain full information on the vibrational spectra.

Raman spectroscopy has some capabilities that are not available in IR spectroscopy [*7*]. For instance, the capability to detect a particular group or structure at very low concentration using resonance Raman, conjugation and/or surface

 U.S. government work. Published 2000 American Chemical Society

enhancement effects. The major advantage is that when one of these effects (which are molecular-structure specific) is present in a sample, Raman spectroscopy becomes a highly sensitive analytical tool. In contrast, in IR the same structures can only be detected at much higher concentrations. Moreover, since some Raman effects can be introduced in a sample, such capabilities are quite versatile. For example, resonance Raman effect depends upon the wavelength of excitation; therefore, it is possible to induce this effect by choosing an appropriate excitation wavelength. We have recently used this approach to study TMPs. Contributions of chromophores in the spectra of TMPs were determined using two different wavelengths of excitation [*8*].

Sensitivity to Chromophores

It has been reported that Raman intensity depends upon several factors [*7,9*]. In addition to being a function of the Raman instrument and the concentration of a component in a sample, effects like x-conjugation, resonance/pre-resonance Raman, and surface-enhanced Raman are important [*7*]. Depending upon the type of effect and the degree to which a particular effect is present, Raman intensity can be enhanced up to a million times. In the studies of lignocellulosics, intensity enhancement due to π-conjugation and pre-resonance Raman effects has been detected [*10*]. For example, when the Raman spectrum of black spruce was obtained using the 514.5 nm excitation, as much as 86% of the intensity of the 1595 cm^{-1} band of lignin could be assigned to conjugation and pre-resonance Raman effects [*10*].

To gain a better understanding of the dependence of Raman intensity on molecular structure, especially in the case of lignin substructures, a study was undertaken using lignin models. Some of these models can be classified as chromophores (e.g., stilbene and coniferaldehyde). Models that were (to varying degrees) and were not n-conjugated to the aromatic ring were selected (Fig. 1, models 1-19). These model structures are named in Table I.

Spectra of model compounds in CH_2Cl_2 at the same molar concentration were obtained using the 514.5 nm laser excitation. Results of this study are summarized in Fig. 2 where relative Raman intensity of certain model-bands (peak heights ratioed to peak height at 1420 cm^{-1}; the latter band is due to the solvent) is plotted for various models. For the phenyl-group band at 1595 cm^{-1}, the intensities were corrected for the number of phenyl groups per molecule. However, for the band representing a C=C and/or C=O group, except for model 9 intensity correction for models was not needed (other models contain only one C=C and/or C=O group per molecule). In the case of *o*- and *p*- quinones (models 15 and 16), respectively, the intensities of 1559 and 1670 cm^{-1} bands were used. It is worth noting that in quinones, the coupling of C=C and C=O stretch modes some times makes the assignment of the Raman bands difficult [*11*].

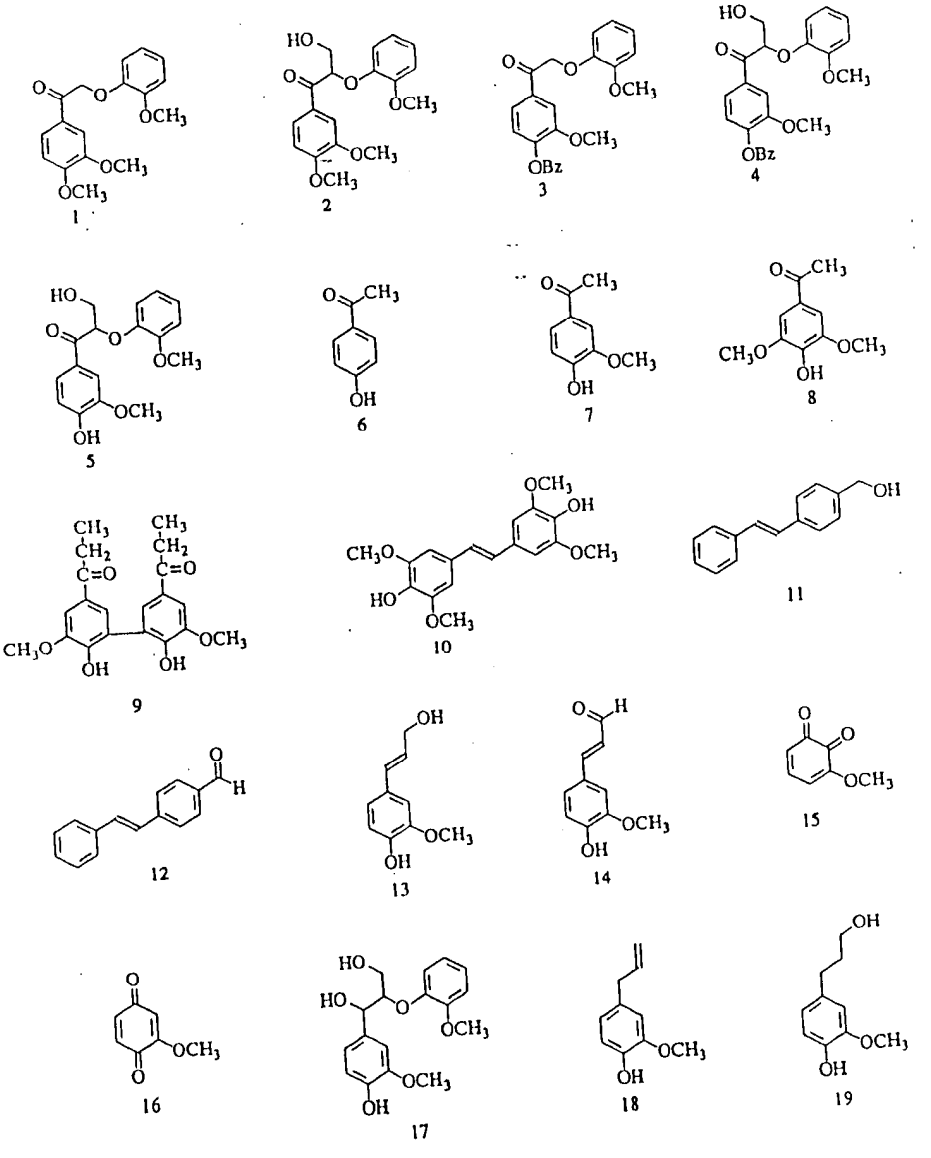

Figure 1. Molecular structures of lignin and chromophore models. See Table I for names of the models.

Table I. Model compounds shown in Fig. 1

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

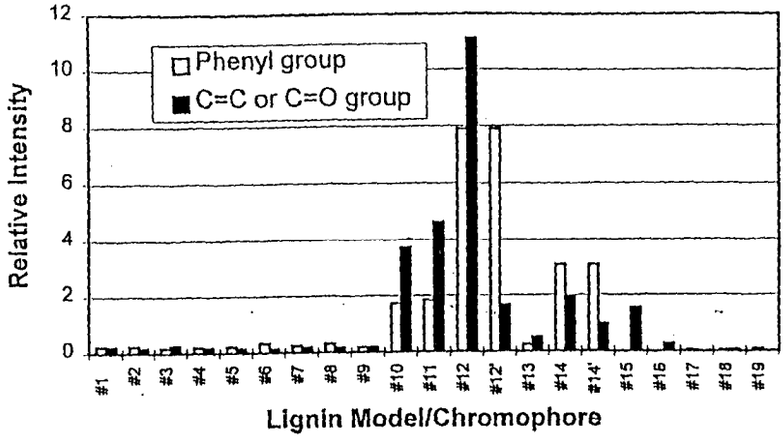

Figure 2. Relative Raman intensities (in 514.5 nm excited spectra) of lignin model compounds (for phenyl, C=O, and C=C groups). Key: see Fig. 1 and Table I. For models 12 and 14, which contain both C=C and C=O groups, #12' and #14' solid bars represent intensities of C=O stretching mode.

From Fig. 2 note that the intensity differences between models were quite dramatic and depended upon the molecular structure. Moreover, for models wherein both the phenyl and C=C or C=O group modes were present the intensity of both modes was enhanced due to the conjugation effect. Considering that the Raman frequency of the phenyl group is similar among various models, the phenyl band is unlikely to be used for characterizing a model. What is needed is a band that is distinct. This requirement is satisfied by the band representing the C=C and/or the C=O bond vibrations (see Table II). The pattern of intensity variation for this vibration (Fig. 2) indicated that different chromophores have different detection sensitivities in Raman. For the models studied, the order of detection, from high to low, was stilbene carboxaldehyde (#12) > stilbene methanol (#11) > syringyl stilbene (#10) > coniferaldehyde (#14) > *o*-quinone (#15) > coniferyl alcohol (#13) > *p*-quinone (#16) > α-keto containing lignin monomers and dimers (#1 - #8) and α-keto biphenyl model (#9) > unconjugated lignin models (#17 - #19).

Raman Spectra

Of the Raman spectra obtained, spectra of *p*-quinones (Fig. 3), methoxy *o*-quinone (Fig. 4), stilbene methanol (Fig. 5), coniferaldehyde (Fig. 6), and methoxy coniferyl alcohol (Fig. 7) are shown in the 850-1850 cm^{-1} region. The displayed wavenumber interval is the region where most prominent features of chromophore models were detected. For the models whose spectra are reported here, the most useful Raman frequencies are listed in Table II.

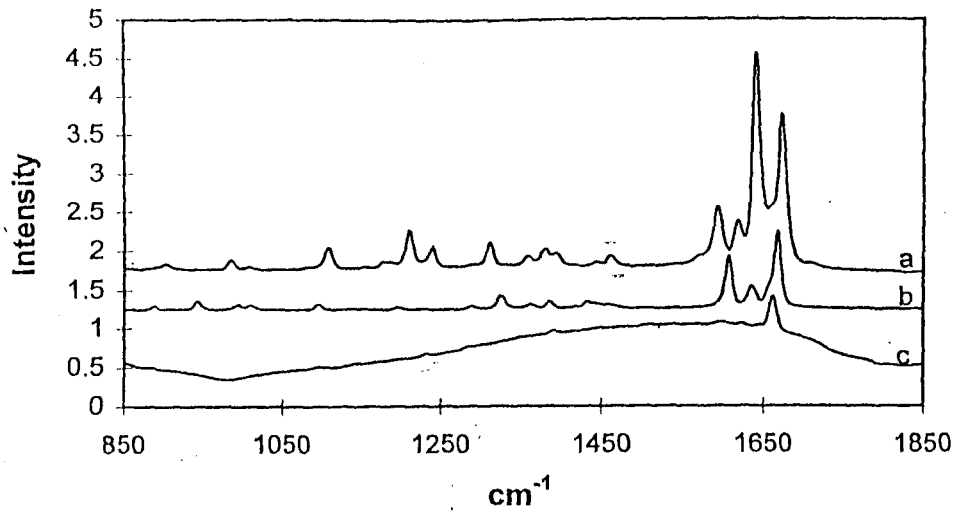

Figure 3. FT Raman spectra of 2-methoxy 1,4-quinone (a), and 2,3-dimethoxy-5-methyl 1,4-quinone (b), and methyl 1,4-quinone (c). The C=O bond vibrations in *p*-quinones have peaks in the region 1665-1680 cm^{-1}.

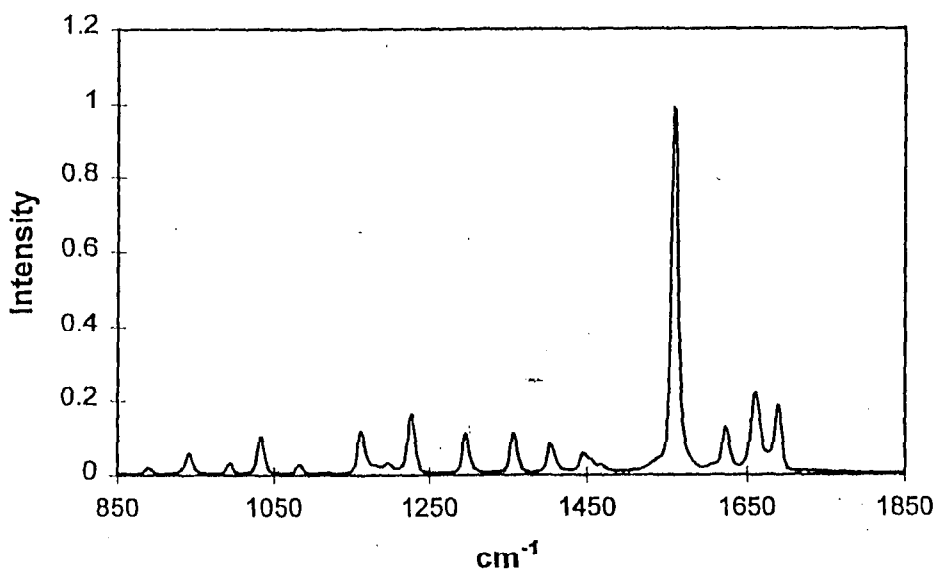

Figure 4. FT Raman spectrum of methoxy *o*-quinone. The strongest band is present at 1559 cm^{-1}.

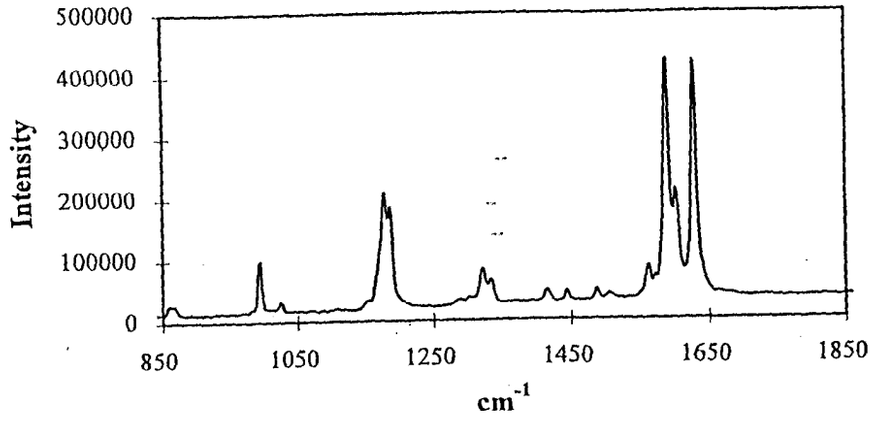

Figure 5. 514.5 nm excited Raman spectrum of stilbene methanol. The C=C band of stilbene is detected at 1635 cm^{-1}.

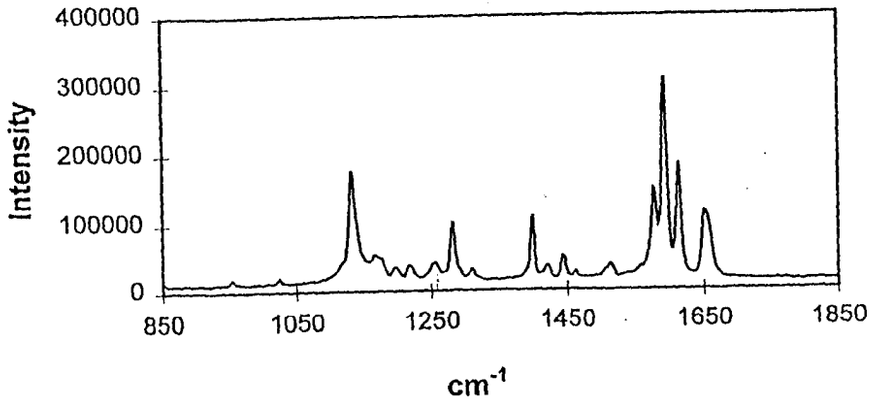

Figure 6. 514.5 nm excited Raman spectrum of coniferaldehyde. Most useful bands are present at 1135, 1595, 1620, and 1654 cm^{-1}.

Table II. Useful Raman frequencies of chromophores[a]

Chromophore	Frequency, cm^{-1}
o-Quinone	1559
Coniferaldehyde	1135, 1620, 1654
Stilbene	1635
Coniferyl alcohol	1654
p-Quinone	1670

[a]Except for the 1135 cm^{-1} band of coniferaldehyde, the bands are due to C=C or C=O groups.

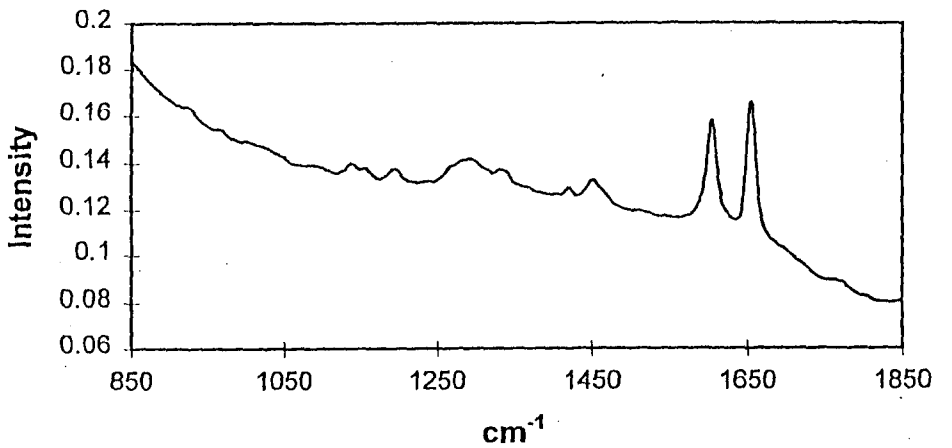

Figure 7. FT Raman spectrum of 4-methoxy coniferyl alcohol. Prominent peaks are detected at 1600 and 1654 cm^{-1}.

From Table II it is clear that, among the models studied, the peak positions of the strongly Raman active modes have vibrational frequencies that are clearly distinguishable. This means that if more than one chromophores was to be present in a sample they all are likely to be detected. Moreover, if on the basis of single-peak-analysis a chromophore group, listed in Table II, was not clearly shown to be present, additional Raman frequencies could be used to facilitate an analysis (Fig. 3-7). This latter approach was used to detect coniferaldehyde structures in mechanical pulps [*1 2*]; in addition to the feature at 1654 cm^{-1}, coniferaldehyde bands at 1620 and 1135 cm^{-1} were used in the analysis.

Applications

Photoyellowing. Light-induced yellowing of lignin-rich mechanical pulps is an area of research that continues to be challenging. Because of the complexity of the phenomenon, however, only limited progress has been made. Recently, using Raman spectroscopy, important information was obtained [*5*,*13*]. Of the many results obtained in our laboratory using Raman, one showed that the chromophores responsible for brightness loss are mostly *p*-quinones [*13*]. More specifically, when thermomechanical pulps were photoyellowed, a new Raman feature at 1675 cm^{-1} was detected [*5*]. In addition to the pulps, the 1675 cm^{-1} feature was present in pulp extracts (yellow) (Fig. 8a, 8b, and 8c). Subtraction of the extracted pulp-spectrum from the unextracted pulp-spectrum clearly showed that the substance removed contributed to the 1675 cm^{-1} band (Fig. 8d).

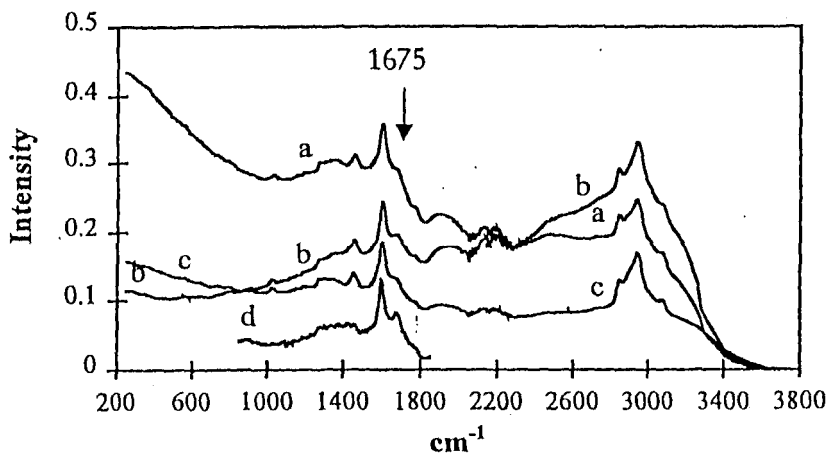

Figure 8. FT Raman spectra of materials extracted from photoyellowed thermomechanical pulps; (a) unbleached, (b) borohydride bleached, (c) diimide reduced, and (d) difference of spectra obtained before and after methanol extraction of a yellowed TMP sample. All spectra show a band at 1675 cm^{-1} which is related to photoyellowing.

Raman spectroscopy played a key role in assigning the 1675 cm^{-1} band to *p*-quinones. When the spectral contribution of yellow chromophores were compared with those of *o*- and *p*- quinones and of Fremy's salt-treated TMP (Fig. 9), it became clear that the contribution at 1675 cm^{-1} was due to *p*-quinone structures [*13*]. This conclusion was also supported by IR analysis of these samples. In Raman spectroscopy, when *o*-quinones and samples containing this functionality are analyzed a band near 1559 cm^{-1} is detected (Fig. 9, spectra d and e). In contrast, for *p*-quinone functionality, a Raman band is expected near 1675 cm^{-1} (Fig. 9, spectra b and c and Table II), Using this information, the band observed at 1675 cm^{-1} in yellowed pulps

was assigned to *p*-quinones. The possibility of the 1675 cm^{-1} band being due to a carbonyl bond stretch (other than *p*-quinone) was ruled out by the fact that this band was quite weak in the IR spectra [*5*]. Carbonyl bond stretch-modes are strong in IR and weak in Raman. On the contrary, in the TMP study, the 1675 cm^{-1} band was strong in Raman and weak in IR.

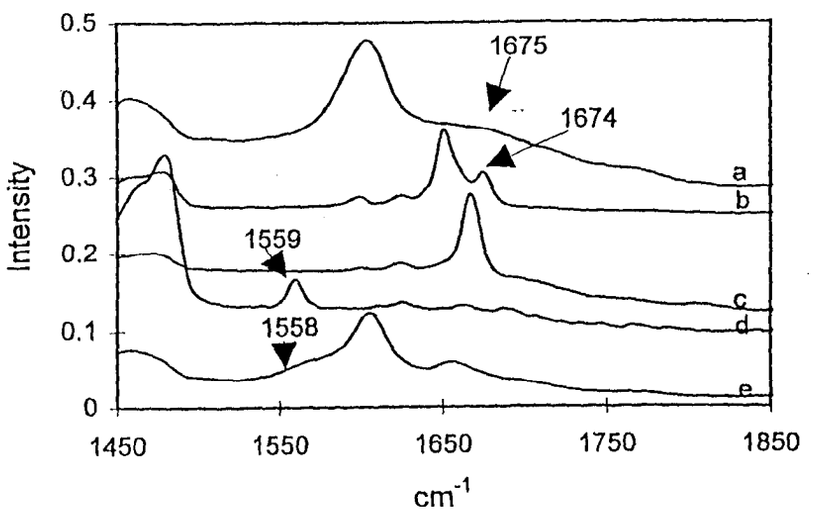

Figure 9. FT Raman spectra of (a) photoyellowed borohydride-bleached thermomechanical pulp, (b) 3-methoxy *p*-quinone, (c) methyl *p*-quinone, (d) 3-methoxy o-quinone, and (e) Fremy's salt-treated, borohydride-bleached TMP. Important band positions are annotated.

Using a' conventional Raman system, laser-induced fluorescence-quenching evidence suggested that both *p*-quinone and hydroquinones were present in mechanical pulp [*13*]. This provided further support that hydroquinone/*p*-quinone redox system was involved in photoyellowing.

Bleaching of Thermomechanical Pulp (TMP). To understand which chromophores are affected when mechanical pulps are bleached using reductive or oxidative bleaching agents, a Raman study was undertaken. Spruce TMP was bleached in accordance with the sequence outlined in Fig. 10. After each bleaching step, the Raman spectrum of the bleached pulp was obtained. This spectrum was compared with the one obtained after the previous bleaching step in the sequence (Fig. 10). In addition, the spectrum of each bleached TMP was compared, with that of unbleached TMP. Pulp brightness is listed in the flow diagram in Fig. 10.

The most prominent changes in the Raman spectrum occurred when the pulp was bleached for the first time with H_2O_2 (Fig. 11, plot c). The resultant changes showed peaks at 1135, 1595, 1622, and 1663 cm^{-1}. These band positions were similar to those reported for the borohydride bleached TMP [*5*]. This suggested that in both reductive and oxidative bleaching the nature of the changes was similar.

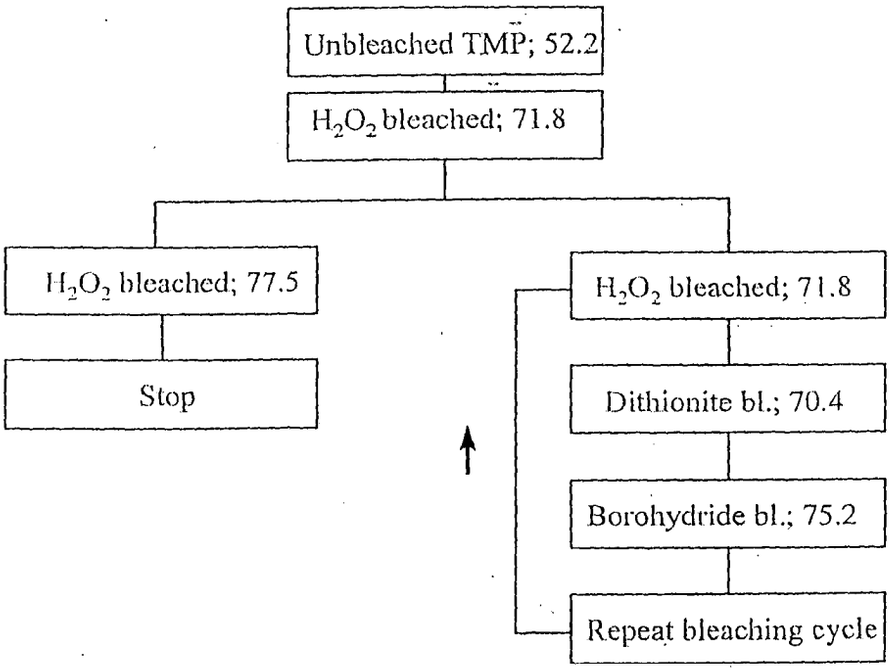

Figure 10. Flow diagram showing sequence of bleaching for, thermomechanical pulp. Brightness values of bleached pulps are also given, Brightness data for pulps bleached the second time (using the repeat cycle) are not shown. These values were 78.8, 79.0, and 79.9, respectively, for H_2O_2, dithionite, and borohydride bleached TMPs.

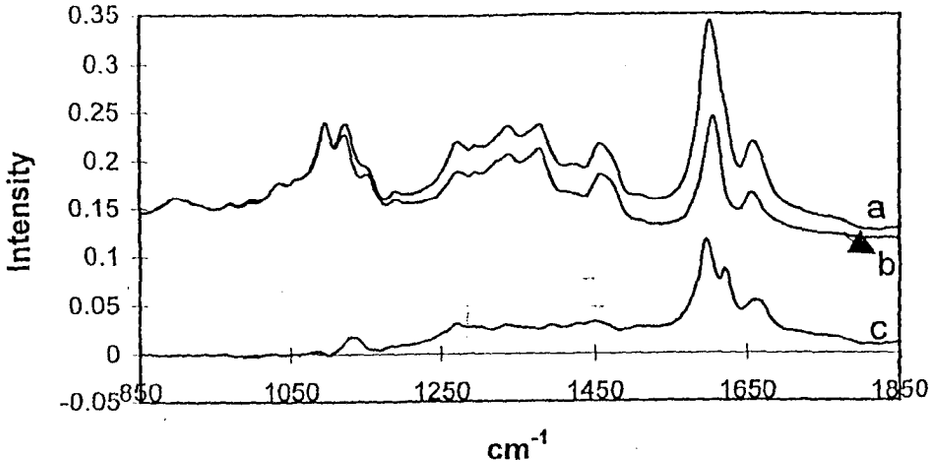

Figure 11. FT Raman spectra of unbleached (a, brightness 52.5) and H_2O_2 bleached (b, brightness 71.8) TMPs. Plot "c" is the difference of unbleached and bleached pulp spectra.

As pointed out previously [5], when TMP is bleached the decline in intensity at 1135, 1595, and 1622 cm^{-1} can be explained by the fact that coniferaldehyde groups are being removed. Moreover, the presence of a peak at 1663 cm^{-1} (in the difference spectrum) can be interpreted in terms of removal of the contributions of both coniferaldehyde and *p*-quinone groups. The C=O stretch in coniferaldehyde has a Raman band at or near 1654 cm^{-1} whereas the *p*-quinones are expected to be in the 1665-1690 cm^{-1} region. Some variation in the vibrational frequencies of coniferaldehyde and *p*-quinones, especially for the latter, is expected because of the dependence of the frequency on molecular structure and intermolecular interactions. This is supported by plot "c" in Fig. 11 where instead of a narrow band a wider contribution in the 1665-1690 cm^{-1} region is detected. Because *p*-quinones entities in TMP are likely to have different molecular structures and different intermolecular interactions, the broadness of the Raman feature can be explained.

After the initial bleaching by peroxide, bleaching with either peroxide or dithionite resulted in only small changes in the spectrum (at 1600 cm^{-1} and in the 1665-1690 cm^{-1} region, Fig. 12 and 13). When spectra of pulps treated with any of the remaining bleaching steps (sequence in Fig. 10) were compared, either no changes or only small changes were detected. This was interpreted in terms of *p*-quinones being oxidized and reduced depending upon the nature of the bleaching agent.

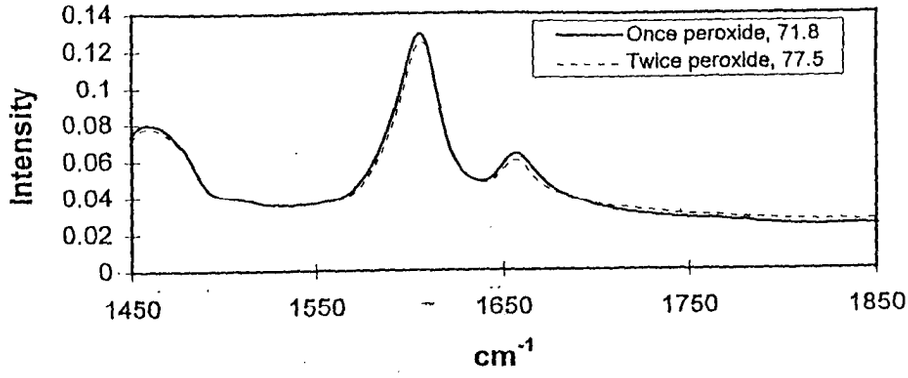

Figure 12. Spectra of once and twice H_2O_2 bleached TMPs. Upon second time bleaching, intensity declined further in the region 1645-1690 cm^{-1}.

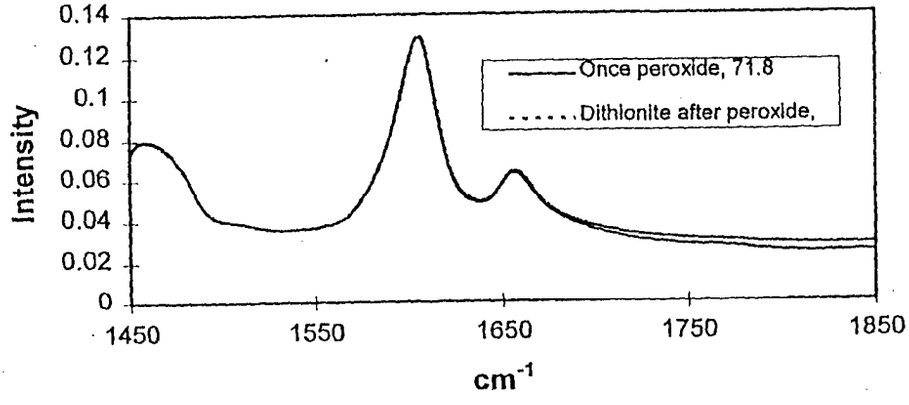

Figure 13. Spectra of once H_2O_2 bleached (dark line) and once bithionite bleached after-once-H_2O_2-bleached (less dark line) TMPs. Upon dithionite bleaching no significant change occurred in the pulp's spectrum.

If *p*-quinones play a major role in determining pulp brightness, as suggested by the Raman analysis, one should be able to correlate brightness with the degree to which these structures are removed upon bleaching. To determine if this occurs pulp brightness was plotted against decline in Raman intensity (in the 1665-1690 cm^{-1} region). As shown in Fig. 14, brightness was found to be positively correlated (linear, regression correlation coefficient 0.89) with the extent of *p*-quinones removed, thus indicating that for TMP, brightness is very likely to be related to the degree to which *p*-quinones groups are present.

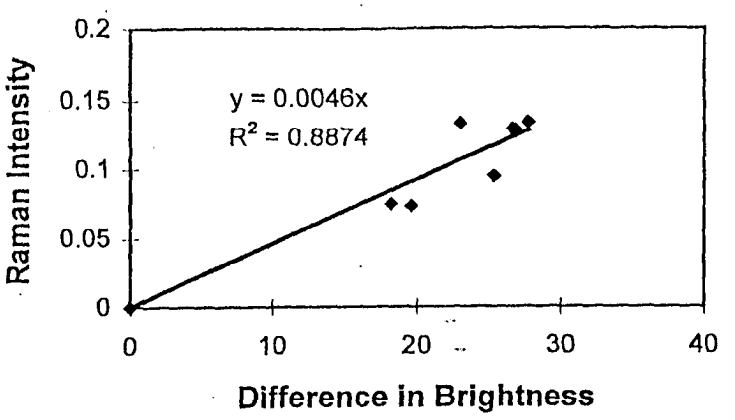

Figure 14. Linear regression between increase in brightness (upon bleaching) and decline in the 1665-1690 cm^{-1} region Raman intensity. The contribution in the 1665-1690 cm^{-1} region is primarily due to *p*-quinones.

Acid Hydrolyzed TMP. It has been reported that peroxide bleached mechanical pulp contain stilbenes [*14*]. This finding was based on HPLC analysis of the acid hydrolysate of peroxide bleached pulp. To investigate whether stilbenes existed in borohydride bleached TMP, the pulp was acid hydrolyzed (using the same method as used in [*14*]) and the pulp and its hydrolysate were analyzed using Raman spectroscopy. Raman was used because its sensitivity to detect stilbenes is extremely good (Fig. 2). If present in any reasonable amount, the contribution of stilbenes should be detected near 1635 cm^{-1} (Table II and Fig. 5). However, Raman spectra of the hydrolyzed pulp and its extract (Fig. 15, plots a and b, respectively) showed no band at or near 1635 cm^{-1}. This indicated that significant quantities of stilbenes were not present in the borohydride bleached pulp.

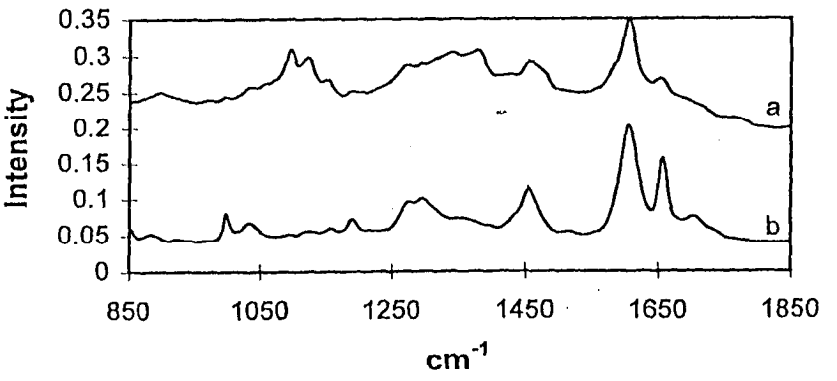

Figure 15. Raman spectra of acid hydrolyzed TMP (a) and extracted material (b). No Raman band at 1635 cm^{-1} was present in either spectrum.

Conclusions

Using Raman spectroscopy, both chromophore and non-chromophore lignin models were studied and it was found that conjugation significantly enhanced the intensity of certain vibrational modes, The spectral information on chromophores was used in the studies of photoyellowing, bleaching, and acid-hydrolysis of TMP. In the area of photoyellowing, the results indicated that *p*-quinones were produced upon light exposure and they are likely to be responsible for most of the brightness loss. Any significant contribution to photoyellowing from stilbenes seems unlikely because these structures 'were not detected. When Raman was used to study bleaching of TMP, evidence suggested that contributions of *p*-quinones were important. Reasonably good correlation between brightness enhancement and the Raman contributions due to *p*-quinones was found. Finally, the technique was used to investigate whether stilbenes were present in a borohydride bleached-TMP. No evidence in support of existence of stilbenes in pulps or its acid hydrolysate was obtained.

Acknowledgments

The authors thank Jim McSweeny for assistance with experiments involving bleaching of pulps. Help received from Sally Ralph in carrying out acid hydrolysis of bleached TMP and in drawing model structures is very much appreciated.

Literature Cited

1. Agarwal, U. P.; Atalla, R. H. Proc. 8th International Symp. Wood Pulp. Chem., Vol. III, **1995,** Gummerus Kirjapainooy, Jyvaskyla, pp. 67.
2. Agarwal, U. P. Fifth Chemical Congress of North America, Cancun, Mexico, **1997,** Book Abstract, Agrochemistry, Abs. # 503.
3. Agarwal, U. P.; Atalla, R. H. *Planta* **1995,** *169,* 325.
4. Agarwal, U. P.; Atalla, R. H. In *Surface Analysis of Paper*; Eds. T.E. Conners and S. Banerjee; CRC Press Inc.: Boch Raton, FL, 1995; Chap. 8.
5. Agarwal, U. P.; McSweeny, J. D. *J. Wood Chem. Tech.* **1997,** *17,* 1.
6. Agarwal, U. P.; Ralph, S. A. *Appl. Spectro.* **1997,** *51,* 1648.
7. Long, D. A. Raman Spectroscopy, McGraw Hill: London, 1977.
8. Agarwal, U. P.; Atalla, R. H. *J. Wood Chem. Tech.* **1994,** *14,* 227.
9. Bernstein, H.J.; Allen G. *J. Opt. Soc. Am.* **1955,** 45, 237.
10. Bond, J.; Ph.D. Thesis, Institute of Paper Science and Technology, Atlanta, GA, 1991.
11. Becker, E.D. J. Phys. Chem. **1991,** 95, 2818.
12. Agarwal, U. P.; Atalla, R. H., Forsskahl, I. Holzforschung **1995,** *49,* 300.
13. Agarwal, U. P. Proc. 9th International Symp. Wood Pulp. Chem., **1997,** Canadian Pulp and Paper Assn., Montreal, Canada, paper K4-1.
14. Gellerstedt, G.; Zhang, L. *J. Wood Chem. Tech.* **1992,** *12,* 387.