4 Vibrational Spectroscopy

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INTRODUCTION

Vibrational spectroscopy is an important tool in modern chemistry. In the past two decades, thanks to significant improvements in instrumentation and the development of new interpretive tools, it has become increasingly important for studies of lignin. This chapter presents the three important instrumental methods—Raman spectroscopy, infrared (IR) spectroscopy, and near-infrared (NIR) spectroscopy—and summarizes their contributions to analytical, mechanistic and structural studies of lignin. The conceptual frameworks used to interpret vibrational spectra are first described in the following section.

VIBRATIONAL SPECTROSCOPY

Vibrational spectra are of two types [1], infrared and Raman, and arise from two different types of energy exchanges between the molecules under study and electromagnetic radiation. In infrared spectroscopy, a vibrational transition that involves a change in dipole moment results in absorption of an infrared photon. The energy of the absorbed photon is equal to the energy difference between the two vibrational states of the molecule.

In Raman spectroscopy, the electromagnetic field induces a dipole moment in the molecule, with the result that an exchange of energy occurs simultaneously with the vibrational transition. The energy of the exciting photons is higher than the energy difference between the two vibrational states, and the exchange with the field results

in a scattered photon, shifted in frequency from the incident photon by an amount equal to the energy difference between the vibrational states.

With both types of vibrational spectroscopy, distinctive spectra and facility in interpretation are possible because only vibrational transitions corresponding to changes in the vibrational quantum number of ±1 are allowed by the spectral selection rules. That is, $\Delta n = \pm 1$, where n is the vibrational quantum number. Due to this, the frequencies observed are usually the fundamental frequencies. In addition, because of analogies between the mathematical descriptions of classical and quantum mechanical vibrating molecular systems, it is possible to rationalize many spectral observations by analogy with classical vibrating systems that possess characteristic force constants and reduced masses. This rationalization has become the basis for systematizing much of the structural and chemical information derived from vibrational spectra.

A useful exception to the primary selection rule is that the overtones and combination bands associated with C–H and O–H stretching vibrations are, in fact, active in infrared absorption. Here $\Delta n = \pm 2$ or ± 3, which results in overtone and combination bands. The apparent violation of the selection rule arises because of anharmonicities in the potential energy function that govern the vibrations. These absorptions occur primarily in the near-infrared region because the selection rules are less rigorous in infrared absorption than in Raman scattering. These transitions are responsible for the spectral features in the NIR. Though the assignment of these transitions to particular vibrational modes is not as easy, the spectral features in this region are very sensitive to compositional variations and provide a valuable analytical tool when they are analyzed using chemometric methods.

In organic molecules, there are well-defined frequencies at which certain bond types of carbon, hydrogen, and oxygen are expected to absorb or scatter [2]. It is therefore possible to correlate frequencies and structures in very useful ways. For example, the most distinctive spectral features are the bands associated with the C–H and O–H stretching vibrations. Since the relatively low mass of the hydrogen atom, the frequencies associated with these stretching vibrations are quite removed from those of the other fundamental vibrational spectral features. Thus, they undergo vibrational transitions as local modes, where the majority of the energy is localized in the particular bond.

It is also anticipated that C=C and C=O double bonds will have distinctive frequencies because these bonds also undergo vibrational transitions as local modes. This is also true of some functional groups, such as carboxyl groups (COOH). In contrast, C–C and C–O single bonds, when adjacent to similar bonds, become involved in a high degree of coupling that can result in bands over a range of frequencies. The high degree of coupling arises because the bonds have similar energies and, therefore, force constants, and they also have similar reduced masses. Thus, skeletal vibrations in an organic molecule tend to be highly coupled, while functional group bands tend to be highly localized.

Application to Lignin

It is important first to consider the types of functional groups that usually occur in lignin [3] and the influence of structural variations on these groups. Two classes of

functional groups are notable. The first class are those that derive their character from a single type of bond and that result in characteristic' absorption (infrared) [4] or scattering (Raman) [5] bands. Here it is expected that the frequencies would occur in a narrow range with slight variations according to the nature of the bonded atoms due to a second-order effect. Good examples of this category are the C-H and O-H stretching vibrations. Their general region (2800 to 3600 cm^{-1}) is determined by the mass of the hydrogen atom; their individual regions are determined by the force constants of the two types of bonds. The C=O bond also fits into this category; the small second-order effects depend on whether it is bonded to a hydrogen atom and carbon atom as in an aldehyde, two other carbon atoms as in carbonyl, or an oxygen atom and carbon atom as in carboxyl. For the C=C bond, the second-order effects would depend primarily on the degree of conjugation in adjacent structures.

The second important class of lignin functional groups consists of clusters of bonds that have collective properties and that derive their identity from the aromatic center. These groups are best discussed in terms of the characteristic vibrations of the aromatic ring and their variation with substitution on the ring. The most complete analyses of aromatic vibrational frequencies, not surprisingly, are those carried out for benzene [6]. Such analyses take advantage of the high symmetry of the benzene ring to carry out detailed normal mode analyses to identify the key internal coordinates contributing to particular vibrational frequencies. These analyses can then become the bases for analyses of the motions of aromatic centers substituted with different functional groups or attached to other substructures. In lignin, the most common functionalities are the variously modified propane substructures that can occur at C1, and the phenoxy, methoxy, and aryl oxygen linkages that can occur at C3, C4, and C5.

In a comprehensive analysis of the vibrational modes that can arise in lignin, Ehrhardt [7] identified the modes that are expected to have a high degree of frequency invariance. They include a cluster of five modes in the aromatic C-H stretching region between 3042 and 3061 cm^{-1}. Another cluster of coupled C-C stretching and C-H bending modes covers the region between 968 and 1594 cm^{-1}; this region includes nine modes. The other modes occur at lower frequencies that may not be readily observed in spectra of lignin. It is anticipated that bands characteristic of the aromatic center will be observed in lignin spectra, although their frequencies will be somewhat altered, and their relative intensities will be significantly different from those observed in the spectra of benzene. The types of modes observed in the spectra of lignin are expected to be similar to those observed in benzene, except that some stretching vibrations associated with bonds to functional groups will not have occurred in the benzene spectra. Moreover, bands that are disallowed in the spectra of benzene because of its high symmetry will not be forbidden in the spectra of aromatic centers in lignin.

Due to its high symmetry, benzene obeys the mutual exclusion rule, so that its infrared active bands are inactive in the Raman spectrum and vice versa. Since the aromatic centers in lignin do not have this symmetry, many more bands are active in both infrared and Raman spectra. However, the pattern remains that the highly polar vibrations are expected to be strongest in the infrared spectrum, whereas the least polar and most polarizable vibrations are expected to be most intense in the Raman spectrum.

The assignments of the spectra discussed in the following sections were undertaken with this background in mind. The analysis by Ehrhardt [7] provided the starting point, but this work was significantly complemented by investigations of the spectra of model compounds. Ehrhardt included studies of mono-substituted and di-substituted model compounds and one tri-substituted model compound. This work has been extended by Agarwal et al. [5, 8–10]to a number of other model compounds that more closely approximate the C_9 units in lignin. The studies included consideration of the various effects that can enhance Raman spectra, as well as the infrared spectra in the fundamental region.

In the following sections, three methods for observing vibrational transitions are outlined together with overviews of their application in studies of lignin and lignocellulosics. The Raman spectral observations are presented in the next section. The instrumental methods applicable to lignin are summarized, the assignments of characteristic bands in the spectra of lignins and lignocellulosics are discussed, and the application in investigations of lignin is outlined. Following the Raman Spectroscopy section, the same classes of information are described for observations based on infrared absorption in the mid-infrared region. This is the region dominated by the fundamental vibrations. Finally, the application of NIR is described. This section does not focus on the assignments, which are not an issue, but rather on the ability to detect subtle variations that are not immediately obvious upon visual inspection. This is accomplished using chemometric spectral analyses.

RAMAN SPECTROSCOPY

BACKGROUND

Raman spectroscopy has existed as an analytical technique for more than 70 years, but its applications to study lignin and lignin-containing materials did not begin until the early 1980s [11–15].A number of factors were probably responsible for this outcome: user unfamiliarity with the technique, the belief that IR and Raman spectroscopy provided similar information (actually they are complementary), and the high cost of Raman instrumentation. Initial spectra [11,13] indicated that the Raman signal was almost completely obscured by laser-induced fluorescence (LIF). Even though a better signal-to-noiseratio could be obtained after acquiring multiple scans and subtracting the background, the spectra were of poor quality. The problem of LIF in lignocellulosics was first reported in 1984 [13]. It arose because lignin absorbed in the visible region, which was used ubiquitously in Raman spectroscopy for sample excitation. It was only after special methods were developed, for both macro [1,11] and micro [1,15] sampling, that the situation improved.

Although a number of visible-laser-based Raman techniques were used to study lignin and lignin-containing materials, a persistent need to suppress the accompanying fluorescence existed. This was true for most nonlignocellulosic materials as well. An important breakthrough in dealing with LIF occurred in 1986 when a new Raman instrument based on NIR excitation was developed [16]. This advance solved the problem of fluorescence for most samples and greatly revived interest in Raman spectroscopy. NIR Raman spectroscopy (also called NIR FT-Raman) has proven

to be an effective technique in lignin research [5] and is rapidly becoming a widely used tool for analysis.

INSTRUMENTATION

Information on obtaining a Raman spectrum is abundant in the literature [17-19]. Raman instruments are either of a dispersive type [17,18] or are based on an interferometer [19]. A dispersive instrument consists of a source of monochromatic radiation (laser), an appropriate way of sampling, suitable gratings (for dispersion of the scattered radiation), and a detection device. In the past, gas lasers were the most common radiation sources; more recently, diode lasers are used. Commercial spectrometers consist of single, double, or triple monochromators, depending upon how efficiently the intensity of the excitation laser line needs to be suppressed to detect the weakly scattered Raman light. In a dispersive Raman spectrometer, a detector consists of either a photomultiplier tube or some multichannel device (e.g., charge-coupled device or photodiode array). A multichannel detector is particularly useful where high resolution is not necessary.and rapid analysis is desired.

In 1986, a Raman instrument based on NIR excitation (1064 nm) and a Michaelson interferometer became available [16]. This development revolutionized Raman spectroscopy. In addition to the advantages of throughput and multiplex inherent to Fourier Transform (FT) techniques, this instrument overcame the obstacle of fluorescence. Fluorescence was eliminated by excitation at a NIR wavelength where electronic transitions in most samples are absent. Availability of such NIR FT-Raman instruments was particularly useful in the studies of lignin.

SPECIAL TECHNIQUES AND EFFECTS

Micro Raman

Raman microspectroscopy couples an optical microscope to the conventional Raman spectrometer [20]. The main advantage of this technique is that a sample can be investigated in a spatially resolved manner. This capability is especially important for heterogeneous materials (e.g., woody tissue) where composition and structure at the microscopic scale can be investigated. In the case of woods, chemical information from the morphologically distinct regions can be obtained. Depending upon the excitation wavelength and microscope-objective characteristics, lateral spatial resolution of the order of 1 μm has been achieved. Most Raman microscopes produced today are also confocal (do not detect out-of-focus scattering), which means that experiments requiring axial resolution (e.g., 2 μm at 633 nm) can be performed. Raman microspectroscopy has been used in investigations of the ultrastructure of woody tissues and other lignocellulosic materials.

Raman Imaging

Raman imaging maps the spatial distribution of a component in a sample, using a Raman frequency that is component-specific [21]. An image is produced using the Raman scattered photons at this frequency. Single-element detectors are used to create line (1D) images and multichannel detectors are used for 2D images. As

useful advances in Raman imaging have occurred only recently, only few reports of applications to lignin-containing materials exist in the literature [22,23].

Resonance and Preresonance Raman

When a compound has an absorption band close to the sample excitation wavelength, either resonance Raman or preresonance Raman scattering occurs [17]. Compared to normal Raman scattering, the resonance Raman effect enhances scattering by as much as a million times. On occasion, overtones of a vibrational mode may be detected. Considering that laser wavelengths are available over most of the absorption frequency region (from NIR to ultraviolet (UV)), the effect can be observed by selecting an appropriate excitation wavelength. Although the preresonance Raman effect was previously reported to be present in the spectrum of native lignin [24], rigorous resonance Raman was observed in residual lignin only in 2001 [25], using UV excitation.

Conjugation Effect

The Raman intensities of certain vibrations depend upon conjugation [26]. Conjugation is defined as the presence of delocalized molecular orbitals caused by overlapping of adjacent atomic orbitals. In chemistry, the concept of conjugation has been used in determining bond lengths and the extent of π-charge transfer between groups of atoms. Raman studies of lignin model compounds indicated that the conjugation effect significantly enhanced the intensities of certain vibrations [9]; for instance, conjugated benzene ring modes, conjugated C=C bond stretching, and conjugated C=O bond stretching. The enhancement seemed to depend upon the extent of conjugation. The presence of conjugated structures in lignin has implications for quantitative work because conjugated structures contribute disproportionately (on a molar basis) to band intensity.

Surface Enhanced Raman

Another way to enhance the usually weak Raman scattering signal of a compound is by introducing the surface enhanced Raman (SER) effect [27] through the influence of small metal particles (usually silver, gold, or copper). In the SER technique, first reported in 1973, the enhancement effect depends upon the nature of the surface roughness and the chosen metal. The mechanisms that lead to the SER effect remain a subject of discussion [28] but are thought to arise from the interaction of adsorbate (chemical effect) and surface plasmons (electromagnetic effect). The SER effect has recently been induced in lignin [29]. The technique has the potential for *in situ* analysis.

SPECTRAL INTERPRETATION

No significant differences have been observed between the FT-Raman spectra of native and milled wood lignins (MWLs) from black spruce [30]. This is likely to be true for other lignocellulosics as well. Therefore, a MWL Raman spectrum can be considered to represent native lignin.

FT-Raman spectra of black spruce [30] and aspen [31] MWLs (representing guaiacyl and guaiacyl-syringyl lignin, respectively) are shown in Figure 4.1, and the

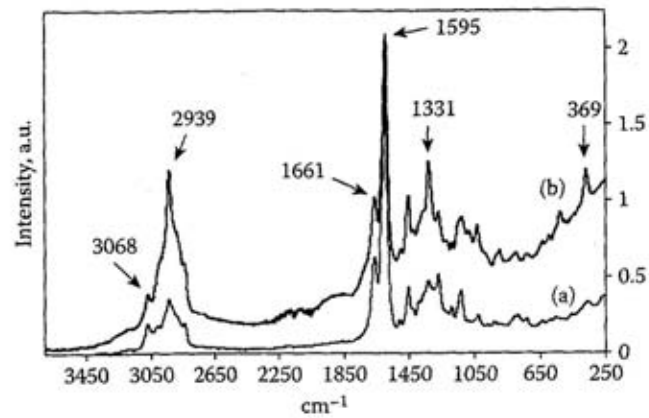

FIGURE 4.1 FT–Raman spectra of milled wood lignins (MWLs): (a) black spruce, (b) aspen. Annotated peaks are some of the peak positions in the spectrum of aspen MWL. Although the spectra appear to be similar, differences between peak positions and intensities were detected (Table 4.1). (Based on UP Agarwal and SA Ralph. FT-Raman Spectroscopy of Wood Identifying Contributions of Lignin and Carbohydrate Polymers in the Spectrum of Black Spruce (Picea mariana). *Appl. Spectrosc.* 51, 1648–1655,1997; UP Agarwal, JD McSweeny, and SA Ralph. An FT-Raman Study of Softwood, Hardwood, and Chemically ModifiedBlackSpruceMWLs. 10thInternationalSymposiumonWoodandPulpingChemistry, Yokohama, 2, 136–140,1999.)

detected band frequencies are listed in Table 4.1. Relative intensities in the table are given with respect to other peaks in the spectrum.

Band assignments are based on the authors' previous work as well as the work of others in the field of vibrational spectroscopy. Useful information was obtained from the spectra of woods [30,32] mechanical pulps [33–36], MWLs [30,31,37], dehydrogenation polymer (DHP) lignins [38] and many lignin models [7,8,10]. Moreover, literature on vibrational assignment of benzene derivatives [2,6] was consulted. Except for the spectral assignments in the region below 925 cm^{-1}, where coupled modes involving deformation and torsional vibrations are present (vibrations of C–C–C, O–C–C, C–O–C, C–O–H, and aromatic ring deformation modes), the assignments have proven quite useful.

Displayed spectra were not processed in any way except that they are shifted on the y-axis with respect to one another for clarity. Contributions from O–H stretching and ester group modes were not detected because their Raman intensity is very weak. This is in keeping with the expectation that polar bonds are not easily detected in Raman spectroscopy because they have poor scattering cross-sections. Also, compared with IR spectra, fewer intense peaks were detected in Raman and they did not overlap as much.

3100–2800 cm^{-1}

In lignin, both aromatic and aliphatic C–H stretches are expected to contribute in this region. The medium intensity peak present at 3071/3068 cm^{-1} (the frequencies are indicated as spruce/aspen) is due to aromatic C–H stretches (Table 4.1). This is supported by the studies of benzene derivatives [2,6,7] and lignin models [10].

TABLE 4.1

Assignment of Bands in FT-Raman Spectra of Softwood and Hardwood Milled-Wood Lignins

Guaiacyl L.[a] (cm^{-1})	Guaiacyl-Syringyl L.[b] (cm^{-1})	Assignment[c]
3071 m[d]	3068 m	aromatic C–H stretch
3008 sh	3003 sh	C–H stretch in OCH_3, asymmetric
2940 m	2939 s	C–H stretch in O–CH_3, asymmetric
2890 sh	2893 sh	C–H stretch in R_3C–H
2845 m	2847 sh	C–H stretch in OCH_3, symmetric
1662 s	1661 s	ring conj. C=C stretch of coniferyl/sinapyl alcohol; C=O stretch of coniferaldehyde/sinapaldehyde
1621 sh	1620 sh	ring conjugated C=C stretch of coniferaldehyde/sinapaldehyde
1597 vs	1595 vs	aryl ring stretching, symmetric
1508 vw	1501 vw	aryl ring stretching, asymmetric
1453 m	1455 s	O–CH_3 deformation; CH_2 scissoring; guaiacyl/syringyl ring vibration
1430 w	1426 w	O–CH_3 deformation: CH_2 scissoring; guaiacyl/syringyl ring vibration
1392 sh	1395 sh	phenolic O-H bend
1363 sh	1367 sh	C–H bend in R_3C–H
1334 m	1331 s	aliphatic O–H bend
1298 sh	---[e]	aryl-O of aryl-OH and aryl-O–CH_3; C=C stretch of coniferyl alcohol
1272 m	1272 m	aryl-O of aryl-OH and aryl-O-CH_3; guaiacyl/syringyl ring (with C=O group) mode
1226 vw	1224 w	aryl-O of aryl-OH and aryl-O–CH_3; guaiacyl/syringyl ring (with C=O group) mode
1192 w	1190 w	a phenol mode
---	1156 sh	unassigned
1136 m	1130 m	a mode of coniferaldehyde/sinapaldehyde
1089 w	1088 w	out of phase C–C–O stretch of phenol
1033 w	1037 m	C–O of aryl-O–CH_3 and aryl–OH
975 vw	984 sh	CCH and -HC=CH- deformation
928 vw	918 sh	CCH wag
895 vw	899 w	skeletal deformation of aromatic rings, substituent groups, and side chains
787 w	797 w	skeletal deformation of aromatic rings, substituent groups, and side chains
731 w	727 w	skeletal deformation of aromatic rings, substituent groups, and side chains
637 vw	638 w	skeletal deformation of aromatic rings, substituent groups, and side chains
---	597 m	skeletal deformation of aromatic rings, substituent groups, and side chains
588 vw	588 w	skeletal deformation of aromatic rings, substituent groups, and side chains
557 vw	---	skeletal deformation of aromatic rings, substituent groups, and side chains
534 vw	531 m	skeletal deformation of aromatic rings, substituent groups, and side chains
---	522 sh	skeletal deformation of aromatic rings, substituent groups, and side chains
---	503 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
491 vw	490 vw	skeletal deformation of aromatic rings, substituent groups, and side chains

(Continued)

TABLE 4.1
Assignment of Bands in FT-Raman spectra of Softwood and Hardwood Milled-Wood Lignins (Continued)

Guaiacyl L.[a] (cm^{-1})	Guaiacyl-Syringyl L.[b] (cm^{-1})	Assignment[c]
---	472 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
457 vw	461 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
---	447 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
---	431 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
---	417 vw	skeletal deformation of aromatic rings, substituent groups, and side chains
384 w	---	skeletal deformation of aromatic rings, substituent groups, and side chains
361 w	369 m	skeletal deformation of aromatic rings, substituent groups, and side chains

[a] Frequencies of black spruce MWL.
[b] Frequencies of aspen MWL; 43% of units are syringyl type.
[c] From UP Agarwal, SA Ralph, and RH Atalla, FT Raman Spectroscopic Study of Softwood Lignin, 9th International Symposium on Wood and Pulping Chemistry, Montreal, 1997.
[d] Note: *vs* is very strong; *s* is strong; *m* is medium; *w* is weak; *vw* is very weak; and *sh* is shoulder. Band intensities are relative to other peaks in spectrum.
[e] No corresponding band in guaiacyl-syringyl lignin

The contributions present at 2845/2847 cm^{-1}, 2890/2893 cm^{-1}, 2940/2939 cm^{-1}, and 3008/3003 cm^{-1} are likely to arise from aliphatic c0H stretches. Considering the types of C-H groups present in the structure of lignin, it is proposed that the bands at 3008/3003 cm^{-1} amd 2940/2939 cm^{-1} are due to the asymmetric C-H stretches in O-CH_3 groups. This is supported by published assignments [2,6,7] and our studies of lignin model compounds [10]. The C-H stretch in R_3C-H structures is expected to contribute at 2890/2893 cm^{-1}. Since several types of these structures are in lignin, small wavenumber shifts can also be expected in the frequency of this mode. Finally, based on the literature [2,6,7] and lignin model work [10], the peak at 2845/2847 cm^{-1} can be assigned to the symmetric C-H stretch in the o-CH_3 group.

1800-1500 cm^{-1}

This is the most informative region of the Raman spectrum of lignin-contributions due to aromatic rings, ethylenic C=C, α- and γ- C=O, and some other chemical groups are detected here. Earlier work with woods [30,32], mechanical pulps [33-36], and MWLs [30,31,37] was useful in assigning the 1662/1661 cm^{-1} band tp the ethylenic C=C (in coniferyl alcohol/sinapyl alcohol units) and γ-C=O (in coniferaldehyde/sinapaldehyde) bond stretches in lignin. Similarly, the 1621 cm-1 shoulder was associated with the ring-conjugated C=C bond stretch (in coniferaldehyde/sinapaldehyde). The aromatic ring stretch modes of lignin were detected at 1600/1595 and 1508/1501 cm^{-1}. Unlike IR, where the 1508/1501 cm^{-1} band is strong, this mode is very weak in Raman.

1500–1000 cm^{-1}

In this region, several bands may represent mixed vibrations because many of the modes are coupled. In addition, there may be overlap as a result of CH_3, CH_2, and CH bending modes. The bands at 1453/1455 cm^{-1} and 1430/1426 cm^{-1} are assigned to O–CH_3 deformation and CH, scissoring modes. In addition, some scattering is expected from the guaiacyl/syringyl-ring vibration. Phenolic O–H bending is thought to be responsible for the shoulder at 1392/1395 cm^{-1}. C–Hbending in R_3C–H structures is likely to give intensity to the feature at 1363/1367 cm^{-1}. Aliphatic O–H bending seems to be the dominant contribution at 1334/1331 cm^{-1}. This feature is stronger in syringyl-rich aspen lignin (Figure 4.1). The triplet at 1298/--- cm^{-1}, 1272/1272 cm^{-1}, and 1226/1224 cm^{-1} (Table 4.1) is likely to have contributions from aryl-O (in both aryl-O–H and aryl-O–CH_3) stretches. In addition, the 1272/1272 cm^{-1} band intensity is in part due to the guaiacyl/syringyl ring (with C=O group) breathing. The peak at 1192/1190 cm^{-1} is weak and is likely to be associated with the phenolic units in lignin. The medium intensity band at 1136/1130 cm^{-1} is associated with the coniferaldehyde/sinapaldehyde unit, although further assignment within the unit is not yet clear. The weak peak at 1033/1037 cm^{-1} seems to be due to the C–O of aryl-OCH_3 and aryl–OH.

1000–350 cm^{-1}

The lowest frequency region of the lignin Raman spectrum is difficult to assign because the number of contributions from skeletal vibrations is likely to increase. The bands are assigned to the skeletal modes of aromatic rings, substituent groups, and side chains. This includes out-of-plane vibrations of these structures. The aspen lignin band present at 369 cm^{-1} is stronger than the 361 cm^{-1} spruce lignin band (Figure 4.1 and Table 4.1). This difference could possibly be due to higher syringyl content (43%) of the hardwood lignin.

UV Resonance Raman Spectra

Lastly, the UV resonance Raman spectra of lignin model compounds were interpreted with the help of partial least squares (PLS) models [39].

APPLICATIONS

lignin in Wood

Several differences have been detected in the FT-Raman spectra of the native lignin in hardwoods and softwoods [30–32,37]. In other research, spectra were used to classify wood type using a mathematical model [40,41]. The model was used for data processing and pattern recognition. In one study [40], a neural computing method was applied to extract key spectral features that were different between the hardwood and softwood species; in another, genetic algorithms were used to classify woods [41]. A chemometric technique called PLS regression has also been used to quantitate constituents, including lignin, of eucalyptus wood [42,43].

From the black spruce study [30], we concluded that the Raman spectra of native, enzyme, and milled wood lignins are very similar. Moreover, we found that

most lignin Raman features were present in regions of the wood spectrum where carbohydrate components did not contribute. These findings are expected to be true for other species of wood as well.

Raman spectroscopy may be useful in determining the syringyl-to-guaiacyl (S/G) ratio in hardwoods. In a study that focused on two species of eucalyptus (samples varied in age and color) [43], a chemometric model was developed for this task and the prediction of the S/G ratio was quite good. Nevertheless, in another study in which a much larger number of hardwoods were sampled and no chemometric approach was used (author's unpublished results), the calculation of S/G ratios was not as accurate. Further work is needed to establish the wider applicability of the Raman/chemometric approach in calculating S/G ratios in wood lignins.

Lignin carbohydrate complexes (LCC) in mangrove, ohirugi [*Bruguiera gymnorrhiza* (L.) Lamk.] and buna [*Fagus crenata* Bl.] were studied using Raman spectroscopy; the LCCs were found to be similar [44].

In a study of fungus-induced changes in lignin responsible for darkening of wood chips [45], the authors concluded that quinones were largely responsible for the brightness loss of the fungus-treated chips.

Studies of native lignin in various morphological regions of wood have been carried out using a Raman microprobe [12,14,22–24,46]. One important result was evidence supportive of lignin orientation in the secondary walls of black spruce [12,14]. Although it was not clear what caused this orientation, this result highlighted the unique information that Raman spectroscopy can provide. Another investigation, which focused on studying corner middle lamellae in white birch and black spruce woods, indicated that the lignin concentration was not constant and could vary by as much as 100% [46]. Using confocal Raman, a small lignified border toward the lumen was observed in the gelatinous (G-) layer of poplar (*Populus nigra* x *P. deltoids*) tension wood [23]. These findings have important implications for understanding the ultrastructure of wood.

Lignin in Mechanical Pulp and Paper

Effects of bleaching [33–36], yellowing (both photo and thermal) [34,35,47], and specific chemical reactions [5,9] on lignin in lignocellulosics have been investigated using Raman spectroscopy. Bleaching studies of softwood thermomechanical pulp indicated that the lignin contained coniferyl alcohol, coniferaldehyde, and *p*-quinone units. The studies further demonstrated that *p*-quinones play a major role in determining pulp brightness [36]. For example, analysis of bleaching-related changes showed that whereas both coniferaldehyde and *p*-quinones were modified upon bleaching, the *p*-quinones were primarily responsible for pulp brightness.

Similarly, in photoyellowing, light-induced changes in lignin structure were ascertained. A time-resolved analysis of thermomechanical pulps showed that the light exposure led to decay of the 1654 cm^{-1} lignin band [5] and was responsible for a new Raman feature at 1675 cm^{-1} [35]. The former band contains contributions from both coniferyl alcohol and coniferaldehyde structures [35,36]. The new band, detected at 1675 cm^{-1} [35], was assigned to *p*-quinones [47]. The conclusion was that exposure of pulps to light caused the decay of coniferyl alcohol and coniferaldehyde units in lignin and caused the formation of *p*-quinones. It is noteworthy that

prior to application of FT-Raman spectroscopy, *in situ* detection of *p*-quinones in photoexposed mechanical pulps was not possible.

Raman spectroscopy was also used to analyze lignin-containing pulps that were chemically modified (sulfonated, acetylated, hydrogenated, methylated, and acid hydrolyzed) [5,9]. In each case, the nature of the change and the extent to which pulp lignin was modified were discerned from the changes in the spectra.

Both lignin-containing and lignin-free coated and uncoated papers have been studied using Raman spectroscopy [48]. In addition to the spectrum of lignin, spectra of the coating mixture components such as latex and $CaCO_3$ were used to analyze these papers [48]. Raman features of coated papers were interpreted in terms of these components; the results showed strong contributions by latex and $CaCO_3$.

Lignin-containing printing and writing papers were aged by light and studied using Raman spectroscopy [49,50]. The changes in the spectrum of lignin for natural and accelerated aging were compared. Based in part on Raman information, experimental conditions for an accelerated test method that simulated natural aging were identified.

FT-Raman spectroscopy was used to differentiate between sulfate and sulfite wood papers [51]. The study indicated that in addition to differences due to hardwood and softwood, sulfite paper spectra showed a band at 510 cm^{-1}.

Residual Lignin in Chemical Pulp

Residual lignin in chemical pulp is difficult to analyze because of its low concentration. However, using FT Raman spectroscopy, the 1600 cm^{-1} band of lignin was easily detected [52–54]. This band has been used to determine pulp lignin content after research showed that its intensity was linearly correlated with pulp kappa numbers. More recently, UV resonance Raman has been applied to study residual lignin [25] with good results. The resonance technique has proven to be highly selective and sensitive for investigating residual lignin. In this case, the content of hexenuronic acid in pulps was determined *in situ.*

Lignin in Other Lignocellulosics

In addition to wood, pulp, and paper spectra, Raman spectra of numerous other lignin-containing materials have been obtained. These include bamboo [55], kenaf, jute, corn, wheat, and sugarcane bagasse [56] and flax [57]. In most cases, information could be obtained on the Raman features of lignin. In the spectrum of bamboo [55], 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 [57].

Another lignocellulose, *Zinnia elegans,* has been analyzed for the presence of cellulose and lignin, and for the effect of the cellulose inhibitor on lignin biosynthesis [58]. Raman information suggested that the inhibitor 2,6-dichlorobenzonitrile has an effect on lignin formation.

Commercial Lignins

Analysis of commercial lignins by Raman spectroscopy remains a challenge. The problem is due the color of the samples, which produces a significant amount

of fluorescence even when the samples are excited at 1064 nm, where most chromophores do not absorb. Since the fluorescence, even longer excitation wavelength is desirable. The other possibility is to modify the chromophores in lignin such that the absorption at 1064 nm is drastically reduced. In principle, it is possible to use resonance Raman and surface-enhanced Raman spectroscopy to study commercial lignins, but research remains to be done to determine if these approaches would be successful.

Chemical Modification Reactions of Lignin

Raman spectroscopy is being increasingly used to monitor chemical reactions [59]. In the chemical industry, online monitoring capability is being developed using fiber-optic-based Raman systems. Our laboratory has obtained useful information on numerous reactions, including bleaching, sulfonation, acetylation, hydrogenation, methylation, and acid hydrolysis.

For example, in photoyellowing, there was a need to determine whether aromatic-ring conjugated coniferyl alcohol C=C groups were completely hydrogenated in mechanical pulps. Using Raman spectroscopy, the hydrogenation reaction was followed by monitoring the intensity decline at 1654 cm^{-1}. Under modified reaction conditions, diimide completely hydrogenated this lignin double-bond in pulp [35].

Acetylation and deacetylation reactions of a mechanical pulp were also monitored using Raman spectroscopy [5]. The most prominent spectral change occurred at 2938 cm^{-1}. The acetylation reaction was considered complete when, upon further acetylation, the intensity of the 2938 cm^{-1} band (relative to 1095 cm^{-1} band) did not change. Analogously, the success of deacetylation was measured by the decline of Raman intensity at 2938 cm^{-1}.

Lignin Quantitation

The lignin spectrum has been used to carry out limited quantitative work. Both the amount of lignin in a sample and the concentration of a specific group within lignin (e.g., coniferyl alcohol) can be determined. The technique was first used to quantify lignin [60], using the 1595 cm^{-1} band, in southern pinewood that was progressively delignified. Due to the presence of a pre-resonance Raman effect, the amount of lignin in untreated wood could not be accurately determined. Nevertheless, a calibration curve for partly delignified samples was obtained. Further quantitative studies have been focused on quantifying lignin in chemical pulps, and the results have been encouraging.

Several factors are important in quantitative work. First, an internal reference band is needed for calculating relative intensity (either area or peak) of the band being used in quantitation. Although an external standard can be used, the internal band-ratio calculation is more reliable. However, if a chemometrics approach (e.g., principal components analysis [PCA], principal components regression [PCR], or PLS) is used, a standard is not required.

Second, considering that Raman intensity depends upon other factors besides concentration (discussed under Special Techniques and Effects), it is important to ensure that these intensity enhancement effects are either absent or their role is minimal. In certain cases, mild chemical treatments can modify a structure to minimize

its enhanced contribution. Another problem in quantitative FT-Raman spectroscopy is "self absorption" [61], which is the absorption of the Raman photons by the sample. As the scattered photons are passing through the sample, they are absorbed and the spectrum obtained is a convolution of Raman and NIR absorption spectra. An investigation specifically designed to determine the importance of self-absorption in lignin and lignin-containing materials [62] found that the bands in the region of 2800–3100 cm^{-1} were reduced in intensity. The decline in intensity depended upon the moisture content of the sample, and the very strong NIR absorption of water beyond 2000 cm^{-1} (when excited by 1064 nm laser) was involved. Self-absorption can be avoided by either performing quantitation using a band that is not affected by self-absorption or by using D_2O instead of H_2O in the sample.

MID-INFRARED SPECTROSCOPY

Background

Mid-infrared spectroscopy (also called FT-IR) has been used to analyze lignins for many years. The mid-infrared (mid-IR) spectral region is the frequency range from 4000 to 400 cm^{-1} (2.5 to 25 μm). Most lignin fundamental molecular vibrations fall in this range. The region below 400 cm^{-1} is the far infrared and that above 4000 cm^{-1} is the near infrared. As the needs of the instrumentation in the far-, mid-, and near-IR regions are different, the techniques in these regions have traditionally been treated differently. The mid- and near-IR differ in sampling techniques as well. Factors such as differences in the absorption in the two regions and the interaction with IR radiation as a function of wavelength are important considerations.

As early as 1948, Jones [63,64] conducted a comprehensive study of lignin using mid-IR spectroscopy. The IR studies by others that followed focused on, for example, synthetic lignin (DHP), lignin *in situ,* Braun's lignin, and enzyme lignin. Hergert [4] reviewed the early research on mid-IR spectroscopy of lignin and summarized the IR band assignments.

Early lignin (solid state) spectra were largely obtained in transmission mode using the potassium bromide (KBr) (or potassium chloride (KCl)) pellet sampling method. Since then, the development of a large number of sampling methods has permitted analytical measurements to be made in reflectance, emission, and photoacoustic absorption modes. New technological advances in instrumentation have benefited FT-IR spectroscopy as well. For example, IR microspectrometry allows the analysis of a small amount of a substance or of a small region of the material. Use of an imaging-capable IR-microscope in conjunction with two-dimensional (2D) detector arrays has allowed production of IR images of materials. Two-dimensional infrared (2 D-IR) spectroscopy, which records spectra at different levels of an applied external perturbation, has also been recently used to study lignin-containing materials.

Advances in data manipulation and availability of mathematical, statistical, and chemometric analytical software programs have greatly assisted extraction of useful information from IR spectra. This area, in particular, has greatly benefited from the wide spread use of personal computers.

In another review of lignin mid-IR spectroscopy [65], band assignments of guaiacyl, guaiacyl-syringyl, and coumaryl–guaiacyl-syringyl type milled wood lignins (MWLs) were summarized.

INSTRUMENTATION

Most modern, commercially available mid-IR instruments are built around an interferometer and are different in this respect from their predecessor, the diffraction IR spectrometer. A modern instrument consists of a source of IR radiation, an interferometer, a sample chamber, and a detector. Brief comments on various spectrometer components are provided in the following text. Details of topics related to instrumentation can be obtained from the literature [66].

As a source, most manufacturers use either a conducting ceramic or a wire heater coated with the ceramic. When heated, these devices emit IR radiation. Temperatures of 1000°C are fairly typical. The interferometer is the heart of the instrument as it analyzes the infrared radiation and enables generation of a spectrum. The Michelson interferometer is the one used most commonly. A computer is used to control the interferometer. Using the fast Fourier transform method, the interferogram is converted into wavelength absorbances. The detectors used in FT-IR instruments are photo resistors — they have very high resistance in the dark, but resistance falls upon exposure to light. Most frequently, deuterated triglycine sulfate (DTGS) is used as the pyroelectric detector of mid-IR radiation. For enhanced sensitivity, a cryogenically cooled mercury cadmium telluride (MCT) semi-conductor detector is used. A computer (data system) is used not only to control the interferometer and perform Fourier transform on collected data, but also to process the data to obtain the most useful information.

SPECIAL TECHNIQUES/INTERFACES

Although mid-IR spectroscopy can be applied to any kind of material in any physical state, sample preparation is an important consideration in FT-IR. For routine analysis of solid lignin or lignin-containing samples, the KBr transmission method is generally used. This sampling approach involves preparation of a compressed KBr pellet containing the sample in a manner that minimizes light scattering. Sometimes, solid samples should be analyzed directly to obtain the desired information. Occasionally, only information from the sample surface is desired. There are special methods to generate such information.

Diffuse Reflectance

Diffuse reflectance infrared Fourier transform (DRIFT) spectra are obtained when IR radiation is incident on a scattering sample at a specific angle and is reflected at all angles. The diffuse reflectance process involves transmission, scattering, and reflection [67]. The technique is used to analyze an intact lignin sample without modification [65]. To study a sample by DRIFT, the sample is either dispersed in KBr (e.g., MWL) or is analyzed directly (e.g., paper sheet) and placed at the focal point of the diffuse reflectance accessory. The scattered light from the sample is collected

by a concave mirror and directed to the detector. Information in the spectrum is dominated by contributions from the surface. However, optical effects, occurring at the surface, can significantly affect the quality of the spectrum. For powdered samples, particle size is important. Further information on the DRIFT sampling method and its applications to study of lignin can be obtained elsewhere [65,68].

Attenuated Total Reflection, or Internal Reflectance

Like DRIFT, attenuated total reflection (ATR) [69] is used to study materials that are difficult to analyze by absorption methods, such as thin layers on nontransparent substrates, substances with very high absorption that are difficult to prepare as thin layers, and materials in which surface sampling needs to be carried out. The principle of measurement is based on the transmission of light through an optical element of high refractive index material (e.g., zinc selenide). The angle of incident light is such that it results in internal reflection. The geometry of the ATR element generates multiple internal reflections. When an IR-absorbing material is on the air-ATR element interface, the light and sample interact and the intensity of the internally reflected light is weakened. A spectrum is produced from the first one or two micrometers (into the sample) from the interface. Further information is available in the literature [69].

IR Microscopy

Infrared microspectrometry has been available for some time [70,71]. This accessory has made it possible to view the sample and select a specific region for chemical analysis. This is of particular interest for materials like wood where spatially resolved (10 μm or larger) information from chemically inhomogeneous regions is desired. In addition, an IR microscope provides high sensitivity and is very useful for studying small amounts of samples (e.g., single fibers, picograms of a substance, and specks in paper sheet). Compared to conventional DRIFT, micro-transmission mode was reported to be more sensitive in studies of kraft pulps [72]. No sample preparation is required and samples are studied directly.

Chemical Imaging

IR imaging is possible by coupling an IR microspectrometer (spectral information) with the focal plane array (FPA, spatial information) [73]. The introduction of FPA detectors allowed the mapping of a sample in practical time because thousands of detector elements are read during a spectral acquisition. Moreover, large sample areas can be mapped by using a programmable microscopic stage. Once obtained from a sample region, thousands of spectra can be evaluated simultaneously (by various ways). This means that a plot of single IR band intensity, representing a single component in a multicomponent sample, can be generated. Such a plot shows how that component is distributed in the sample. Such chemical information can be converted into an image and then compared to the visual image [74].

2-D IR

Introduced around 1990 [75,76], 2-D IR is a technique in which an external perturbation (e.g., temperature, pressure, or strain) is applied to the sample and time-dependent IR

spectra are recorded. A 2-D correlation spectrum is then produced, plotting v_1 and v_2 (bands at two wavenumbers) in the two dimensions; the third dimension shows the correlation function of the spectral intensities observed at v_1 and v_2. The shape of the resulting surface shows whether or not the bands at the two wavenumbers are correlated. This allows assessment of the extent to which two parts of a molecule are linked (coupled) in their response to the applied perturbation. This technique has only recently been applied to the study of lignin [77].

Photoacoustic IR

Photoacoustic IR (PAS-IR) [78,79] involves direct measurement of the absorption of IR radiation. This spectroscopic technique is based on the conversion of absorbed infrared radiation into thermal energy, followed by the emission of sound produced by the thermal energy transfer from the sample into the surrounding gas phase. Carbon black is used as a reference material because of its excellent absorption characteristics. It is a good method for difficult-to-analyze samples. The technique has been applied to the study of lignin [SO,Sl].

Transient IR

Transient infrared spectroscopy (TIRS) is a mid-infrared technique [82] that has been developed to obtain spectra of moving solids and viscous liquids. TIRS spectra are obtained from the generation of a thin, short-lived temperature differential that is introduced by means of either a hot or cold jet of gas. When a hot jet is used, an emission spectrum is obtained from the thin, heated surface layer. This technique is known as transient infrared emission spectroscopy (TIRES). When a cold jet is used, the blackbody-like thermal emission from the bulk of the sample is selectively absorbed as it passes through the thin, cooled surface layer. The result is a transmission spectrum convoluted with the observed thermal spectroscopy. This method is known as transient infrared transmission spectroscopy (TIRTS). TIRS is ideally suited for online analysis because it is a single-ended technique that requires no sample preparation. This technique has been applied to the lignin analysis of wood chips [83].

Fiber Optic Mid-IR Spectroscopy

Using optical fibers, mid-IR spectroscopy has been used for online analysis and remote sampling [84]. Fibers used in the mid-IR region are produced from oxides, chalcogenides, and halides of various elements. To be useful, the fibers must have IR radiation transmission capability over short distances. Like ATR crystals, fibers are based on the total reflection of radiation inside a material.

SPECTRAL INTERPRETATION

Since mid-IR spectroscopy has been used for a long time in lignin analysis, the IR features of lignin have been studied extensively. Most bands have been previously assigned [4,65], and it has been reported that the coupling of different vibrational modes exists in the spectra. Spectra of black spruce and aspen MWLs are typical of softwood and hardwood MWLs (Figure 4.2). These spectra were obtained in the

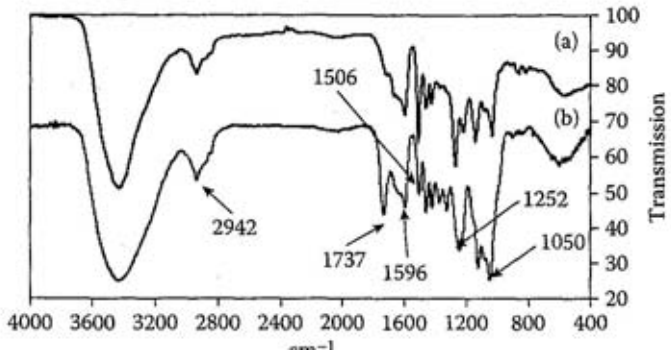

FIGURE 4.2 FT–IR spectra of MWLs obtained in the authors' laboratory: (a) black spruce; (b) aspen. Some IR bands in the aspen spectrum are annotated. The spectra differ with respect to band position, intensity, and shape.

authors' laboratory. Since there are wide variations in wood lignin structure and composition, wood spectra have significant differences (band positions, intensities, and shapes). The band assignments given in Table 4.2 were taken from Hergert [4] or Faix [65] unless otherwise indicated. Readers are referred to this original literature for details of band assignment.

In addition to the band assignments, mathematical approaches such as deconvolution [85,86], band fitting [87], and derivatization [88] have been used extensively in the literature as aids to interpreting spectra. Although these techniques are generally useful, some have limitations or potential for introducing artifacts [87]. The user of these approaches is cautioned and is advised to make informed choices.

Applications

Lignin in Wood

A well-established use of mid-IR spectroscopy is to differentiate hardwoods and softwoods [90], based on the documented differences between hardwood and softwood lignin. It has also been used to quantitatively determine the amount of lignin in woods [83,91–93]. Examples of the use of FT-IR to study modification of wood lignin include weathering [94], photodegradation [95], fossilization [96,97], fungal treatment [98], and chemical reactions [99,100]. In addition, IR spectroscopy has been used in situ to determine functional groups in lignin. Studies of oak ray cell wall [101] and photodegradation were also carried out using infrared microscopy [102].

Lignin in Mechanical Pulps

Lignin-containing pulp and paper samples have been frequently analyzed by FT-IR (for a review see [103]). For instance, bleached [91,104,105], yellowed (both thermal and photo) [91,104–107] and biomechanical pulps [101] have been analyzed. Of the various methods of sampling, DRIFT has been used most often; photoacoustic, ATR, and micro methods have been used sparingly.

TABLE 4.2
Assignment of Bands in FT-IR Spectra of Softwood and Hardwood Milled-Wood Lignins

Guaiacyl L.[a] (cm^{-1})	Guaiacyl Syringyl L.[b] (cm^{-1})	Assignment[c]
3430 vs[d]	3440 vs	O–H stretch, H-bonded
2938 m	2942 m	C–H stretch methyl and methylene groups
2885 sh	2882 sh	C–H stretch in methyl and methylene groups
2849 sh	2848 sh	C–H stretch $O–CH_3$ group[e]
1717 sh	1737 vs	C=O stretch, unconjugated ketone, carboxyl, and ester groups
1667 sh	1670 sh	ring conj. C=O stretch of coniferaldehyde/sinapaldehyde
1645 sh	1643 sh	ring conjugated C=C stretch of coniferyl/sinapyl alcohol
1600s	1596 s	aryl ring stretching, symmetric
1513 vs	1506 vs	aryl ring stretching, asymmetric
1466 s	1464 s	C–H deformation, asymmetric
1458 sh	1425 m	$O–CH_3$ C–H deformation, asymmetric
1428 m	1379 m	Aromatic skeletal vibration combined with C–H in plane deformation
1375 w	1367 sh	$O–CH_3$ C–H deformation symmetric[e]
1331 sh	1330 m	Aryl ring breathing with C–O stretch
1270 vs	1252 vs	Aryl ring breathing with C=O stretch
1226 m	---[f]	C–C, C–O, and C=O stretches
1142 s	1159 sh	Aromatic C–H in plane deformation
---	1127 vs	Aromatic C–H in plane deformation
1085 w	1082 sh	C–O deformation, secondary alcohol and aliphatic ether
1035 s	1050 vs	Aromatic C–H in plane deformation
914 vw	905 w	C–H deformation out of plane, aromatic ring
878 sh	---	C–H deformation out of plane, aromatic ring
863 w	---	C–H deformation out of plane, aromatic ring
823 w	---	C–H deformation out of plane, aromatic ring
784 vw	---	CCH wag, mine from Raman
742 vw	---	skeletal deformation of aromatic rings, substituent groups, side chains[g]

[a] Frequencies of black spruce MWL.
[b] Frequencies of aspen MWL; 43% of units are syringyl type.
[c] From HL Hergert, in *Lignins: Occurrence, Formation, Structure and Reactions,* Wiley-Interscience, New York, 1971; O Faix, *Methods in Lignin Chemistry,* Springer-Verlag, Berlin, 1992, unless otherwise indicated.
[d] Note: *vs* is very strong; *s* strong; *m* medium; *w* weak; *vw* very weak; and *sh* shoulder. Band intensities are relative to other peaks in spectrum.
[e] From W Collier, VF Kalasinsky, and TP Schultz, *Holzforschung,* 51, 1997.
[f] No corresponding band was observed for guaiacyl-syringyl lignin.
[g] From D Lin-Vien, NB Colthup, WB Fateley, and JG Grasselli, *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules,* Academic Press, San Diego, 1991.

Both DRIFT and IR-PAS [81] have been used to investigate chemical changes in pulp lignin caused by the reductive or oxidative bleaching of mechanical pulps. The results showed a decline in aldehyde and ketone C=O band intensity (due to alkaline peroxide and sodium borohydride bleaching) and a reduction in contributions from conjugated carbonyl groups.

Light-induced modification of lignin in mechanical pulps has been thoroughly studied using FT-IR, both in pulps directly and in lignin isolated from yellowed pulps. Photoexposure resulted in the destruction of guaiacyl structures (in a softwood pulp) and led to production of carboxyl and/or ester groups [35,104]. IR-evidence supporting formation of *p*-quinone groups (upon photoyellowing) was also found [35]. Furthermore, the intensity of the 1727 cm^{-1} C=O band was linearly related to the post color number of the pulp [35]. FT-IR spectroscopy was also used to study chemical changes in the depth direction of paper exposed to light [106] on only one side. This study established that light-induced changes occurred to a depth of 50 to 100 μm, and that approximately 50% of the changes occurred at a depth of 20 μm. In addition to evaluating the effects of light- or heat-only treatments, FT-IR was applied to study mechanical pulps that were treated with both light and heat (light followed by heat and heat followed by light) [106].

Biomechanical straw pulps treated with enzyme mediator systems have been studied using mid-IR spectroscopy [108]. IR spectra indicated that a manganese-based enzyme system in combination with hydrogen peroxide generated the glucose oxidase couple, which removed aromatic ring structures.

A method based on an IR technique has been patented for determining lignin content (and other pulp properties) of pulp suspensions [109].

PAS-IR in conjunction with multivariate analysis has been applied to develop useful correlations to predict, among other things, methoxyl content and number of phenolic groups in a sample [110]. Another recently developed technique, 2D IR, was applied to study lignin-rich pulps [77]. The researchers concluded that lignin imparts strong viscoelastic behavior to pulp fibers, and the technique allows the study of lignin orientation.

Residual Lignin in Chemical Pulp

FT-IR has been used for both characterization [111] and quantitation [112,113] of residual lignin in chemical pulps. Due to the low lignin concentration, the infrared band at 1510 cm^{-1} is most suited for the latter purpose. Good correlations between kappa number and IR intensity were found for kraft pulps produced from a range of hardwoods and softwoods. The advantage of mid-IR analysis was that no sample preparation was required.

Isolated Lignins

When lignin is part of a multicomponent sample, it can often be analyzed *in situ*. However, the extent of information obtained is limited because of spectral overlap with other sample components and because the lignin is dispersed in the matrix. A spectrum of much higher quality can be obtained when isolated lignin is analyzed [114]. Depending on the source and the isolation procedure, the IR spectrum of lignin can vary significantly. For example, the spectrum of ball-milled enzyme

lignin (isolated from cottonwood) is quite different from that of kraft indulin (mixed softwood) lignin [115,116]. The latter is isolated from the waste stream of the chemical pulping process and has thus undergone drastic structural changes.

Using IR band position, intensity, and shape, detailed studies of lignin have been conducted and the amounts of various functional groups (e.g., carbonyl, hydroxyl, and phenolic) have been determined. Isolated lignins have also been subjected to various chemical treatments (e.g., oxidation, acetylation, methylation, and oximation) and then analyzed by IR to understand how such treatments modify the IR spectrum of lignin.

In one investigation [117], 2-D correlation mid- and near-IR spectra of isolated lignins were used for qualitative spectral interpretation of the lignin from plant materials.

lignin in Other Lignocellulosics

The determination of lignin content using IR techniques has also been conducted in plants other than wood. For example, the determination of kenaf lignin in plant materials using DRIFT has been described [118]. A linear relationship existed between the peak area at 1506 cm^{-1} and lignin content. The lignin content of four kenaf varieties (by weight of plant material) was 10.4–10.8% in the bark, 20.5–20.6% in the wood, and 14.9–15.3% in the pith.

lignin in Solution

Infrared spectroscopy is seldom used to study lignin in solution, though the need for such a study occasionally arises; for example, for the analysis of spent pulping liquors or soluble lignin fractions. Faix [119] provides a review of sampling accessories and methods as well as information on the type of lignin samples analyzed.

lignin Quantitation

FT-IR has played an important role in chemical analysis for decades. The current availability of numerous chemometric/multivariate techniques has also made IR spectroscopy a tool of quantitative measurement. Mid-IR spectroscopy answers the question of lignin content by performing either univariate or multivariate analysis. In the former case, the intensity of one lignin band is used for calculating the concentration. This is possible because observed IR band intensity is usually a linear function of lignin concentration. In such a calculation, the 1510 cm^{-1} band of lignin has almost always been used. In a multivariate analysis, multiple points in a spectrum are measured; this is done for each sample with varying concentration of lignin. Such data have been used to develop mathematical and statistical models capable of accurately predicting lignin concentration. The end goal of relating spectral changes to lignin concentration has thus been achieved.

NEAR- INFRARED SPECTROSCOPY

BACKGROUND

The region between the infrared and visible regions, from 4000 to 12500 cm^{-1}, is referred to as near-infrared. NIR vibrations of almost all materials are overtones and

combination modes [120,121]. Most vibrations are modes of C–H, O–H, and N–H stretches. In a NIR spectrum, some absorption bands can be correlated with functional groups, although the bands are inherently weak compared to those of a mid-IR spectrum. However, an NIR spectrum also has information on composition, conformation, crystallinity, and inter- and intra-molecular interactions.

A review of NIR applications to lignin studies in 1992 [65] compared NIR spectra of milled softwoods and hardwoods and milled wood lignins (MWLs) (isolated from the same woods). There were numerous unresolved overlapping bands and there were only minor differences between the spectra. In addition, acetylation of MWL caused significant changes in the spectrum [65]. Another review of NIR spectroscopic studies of wood and wood products was published in 1995 [122]. Applications of NIR spectroscopy to lignin range from kappa number determination in chemical pulps [123] to lignin content in wood [124] and plant residues [125].

As for Raman and mid-IR spectroscopy, advances in data manipulation and the availability of chemometric analytical software have greatly assisted extraction of useful information from near-IR spectra. This is particularly important for NIR spectra because they are difficult to interpret in terms of chemical structure. An additional technical advance, fiber optics, has allowed the acquisition of NIR spectra by remote sensing.

INSTRUMENTATION

Early NIR work was performed using either a UV-Vis instrument with extension units for low wavenumbers or IR spectrometers with accessories for high wavenumbers. With these instruments, the quality of the collected spectrum was low. However, in modern times, good quality dispersive- and FT-NIR spectrometers exist that provide high quality spectra.

A NIR spectrometer can be built in a number of optical configurations. Analogous to the configuration of mid-IR, the basic configuration for NIR consists of a light source, a light dispersing element (grating), a sample, and a detector. In the region 1100–2500 nm, a lead sulfide (PbS) detector is usually used, whereas for the region below 1100 nm, PbS sandwiched with silicon photodiodes is used. Within a dispersive instrument, there are two modes of sample excitation, predispersive (light dispersed before falling on the sample) or post-dispersive. Moreover, spectrometers with Fourier transform (FT) capability are available in place of dispersive instruments. However, these spectrometers do not provide a spectrum with high signal-to-noise ratio. Filters (e.g., interference filter, turret-mounted filter) have also been used to obtain NIR radiation at various wavelengths. Another type of filter, the acousto-optic tuneable filter (AOTF), has been used as a diffraction grating. The AOTF relies on the acoustic diffraction of light in an anisotropic medium. Spectrometers are also developed with diode array detectors. Such instruments are very fast because they can detect multiple wavelengths simultaneously.

In the diffuse reflectance mode, two to four detectors can be used. The analog signal is averaged before being digitized. The computer records a signal representing wavelength and the reflectance or transmittance data, for both the sample and the

reference. The final NIR spectrum is the difference between these two reflectance (transmittance) spectra.

Integrating spheres are also prevalent in NIR spectroscopy and have the advantage of improving the efficiency of signal collection. Workman and Burns [126] have reviewed NIR instrumentation.

Special Techniques and Interfaces

Samples are analyzed either in transmission or diffuse reflection mode. The latter is now a widely accepted method in quantitative analysis. Diffuse reflectance measurements penetrate about 1–4 mm of the front surface of samples; in nonhomogeneous samples, shallow penetration is the cause of variation in the spectrum. In transmission, on the other hand, the entire thickness of the sample is measured and error due to nonhomogeneity is minimized. These sampling methods are the same as those discussed in the section on mid-IR spectroscopy. For detailed information on diffuse reflection in NIR, the reader is referred to the literature [127].

Diffuse reflectance NIR spectra of black spruce and aspen MWLs are shown respectively in Figures 4.3 and 4.4. The two spectra are very similar.

Applications

As direct spectral interpretation is limited in NIR spectroscopy, multivariate mathematical methods are used to obtain useful information. These techniques are used to develop mathematical models that correlate spectral features to properties of interest. For quantitative work, calibration models are needed that relate the concentration of a sample-analyte to spectral data. Information on developing calibration models and data analysis is provided elsewhere [128–132].

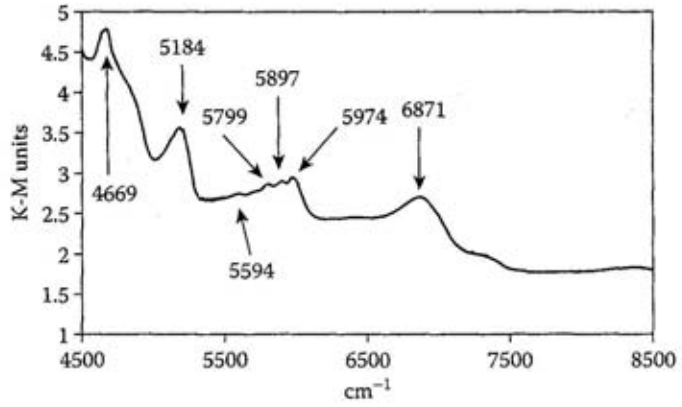

FIGURE 4.3 Near-infrared (NIR) spectrum, obtained in authors' laboratory, of black spruce milled wood lignin (MWL). Most peaks in the spectrum are annotated. The spectrum is similar to that of aspen (see Figure 4.4).

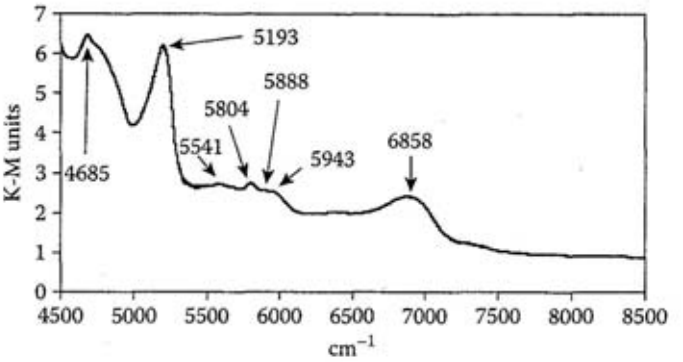

FIGURE 4.4 NIR spectrum of aspen MWL, obtained in authors' laboratory. Most peaks in the spectrum are annotated. The aspen and black spruce (Figure 4.3) spectra are very similar.

Lignin in Wood and Leaves

Schultz and Burns [133,134] applied NIR to determine lignin in pine and sweetgum (softwood and hardwood, respectively) and compared the two IR techniques for this application. They found that NIR was superior for determining lignin [134] with standard errors of calibration 4% over the lignin concentration range of 10–30%. FT-NIR has also been applied for wood identification in an examination of 90 samples of 12 different wood species [135]. The results showed that once the measuring unit had been calibrated, it was possible to identify new samples of the species previously tested. The authors concluded that differentiation between samples of a given wood species of different origins might be possible by NIR spectroscopy. A study for determining lignin content in wood by FT-NIR [136] concluded that this is an accurate, fast, and reliable method. The standard deviation was in the 0.11–0.14% range and root mean square error was <0.345%.

The quality of eucalypt woods for producing chemical pulps was evaluated using NIR spectra and chemometric methods [124]. NIR spectroscopy was used to predict pulp yield and cellulose content from spectra of powdered wood samples [137]. In another application, in addition to estimating lignin content, NIR spectra were used to quantify hardwood–softwood ratios in paperboard [138]. NIR spectra taken from solid European larch samples subjected to axial bending and compression tests revealed an excellent ability to model the variability of mechanical properties [139]. The study demonstrated that the model is based not only on the measurement of density, but also on surface geometry, composition, and, possibly, lignin content. The authors concluded that NIR spectroscopy shows considerable potential to become a tool for nondestructive evaluation of small clear wood specimens, e.g., increment cores.

NIR laboratory data of whole fresh leaves were evaluated with respect to leaf chemical composition [140]. NIR spectra were measured for 211 foliage samples, which included both broad- and needle-leafed species. Multiple linear regression analysis was used to determine if reflectance data from fresh leaf samples contained information on nitrogen, lignin, and cellulose concentrations. Calibration

equations were developed for all leaf constituents, indicating that information on leaf biochemistry is present in the spectra of fresh as well as dried ground leaf samples.

Residual Lignin in Chemical Pulp

NIR spectroscopy has been used to investigate residual lignin and chemical pulps. In a study of delignification, birch chemical pulp was analyzed and a PLS model for Klason lignin was developed [141]. Compared to other methods, NIR was the most reliable (standard error <1%). The range of Klason lignin measurement was 0–25%. In a study of the kappa number of batch kraft pulp, different NIR sampling methods were compared [142]. The best results were obtained with homogenized, dried samples. The authors concluded that kappa could be measured (with a 95% confidence interval) consistently with ±2 kappa numbers. NIR was also used to predict kappa number, hexeneuronic acid, and Klason lignin in hardwood kraft pulp [143]. For the first two parameters, no difference was noted between dry and semidry samples; for Klason lignin, dry samples provided somewhat higher values. Finally, black liquors from alkaline pulping were analyzed and the concentration of the main liquor components (including lignin) were established [144].

lignin in Other Lignocellulosics

NIR spectra of dry ground leaf samples were analyzed to obtain an unmixed spectrum of lignin and other forest foliage components [145]. In another application, NIR was used for remote sensing in the measurement of lignin, cellulose, and nitrogen concentrations in forest canopies [146]. The relative value of near- and mid-infrared diffused reflectance spectroscopy in determining the composition (including lignin) of forages and by-products has been evaluated [147]. Sixty-seven samples consisting of 15 alfalfa, 16 tall fescue, and 15 orchard grass hay samples, 10 corn stover samples, and 11 wheat straw samples at various stages of maturity were examined by these techniques. The results showed that diffuse mid-IR reflectance spectroscopy can perform as well as, and sometimes better than, diffuse NIR spectroscopy in determining the composition of forages and by-products. In addition, FT-NIR spectroscopy did not perform as well as either NIR spectroscopy using a scanning monochromator or FT-IR spectroscopy. Finally, diluting samples with potassium bromide was not beneficial for either of the Fourier-based determinations.

Other lignin applications of NIR included research on flax fiber [148], compost prepared from wheat straw and chicken litter [149], hard red winter and spring wheat [150], and degradation of plant cell walls by white-rot fungi [151].

CONCLUDING REMARKS

Raman, mid-IR, and near-IR techniques have played an important role in lignin analysis. These techniques provide fundamental knowledge at the molecular level, and will likely continue to be used in this way. Recent developments in Raman instrumentation have made this method even more versatile and user-friendly. Specific

techniques such as FT-Raman, resonance Raman, SER, and Raman mapping are all expected to be widely used as compact, easy to use, integrated instruments become commercially available at reasonable cost. Historically, mid-IR spectroscopy has been used most extensively to analyze lignin due to the early development of the techniques, user familiarity and the existence of widely available, low-cost instrumentation. Between mid- and near-IR, the latter continues to see more vigorous developments in instrumentation and applications. Development of diffuse reflectance techniques and application of chemomertic methods have been two major advances in this area. Both are widely used in the quantitative analysis of lignocellulosic materials. The future of the lignin vibrational spectroscopy is bright and stimulating.

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