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Determination of the carbonyl groups in native lignin utilizing Fourier transform Raman spectroscopy

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Abstract

A near-infrared Fourier transform Raman (NIR-FTR) spectroscopic technique was utilized to determine the chemical structure of lignin in a woody matrix. In the NIR-FTR spectra of coniferaldehyde and coniferyl alcohol, the Raman bands for the carbonyl group and the α, β unsaturated bond were detected at 1620 and 1660 cm^{-1}, respectively. These peaks were also found in the NIR-FTR spectra of chemically synthesized lignins, isolated lignin from conifer wood, and conifer wood meal. Upon the reduction of carbonyl groups in the lignin samples and wood meal, the band at 1620 cm^{-1} disappeared; on the other hand, the band at 1660 cm^{-1} remained unchanged. However, upon the oxidation of reduced lignin at the benzyl hydroxyl group using dicyanodichrolobenzoquinone, the band at 1620 cm^{-1} clearly appeared, strongly suggesting that the band at 1620 cm^{-1} can be assigned as a carbonyl marker band. The hydrogenation reaction optimized for the reduction of the unsaturated bond in lignin caused the disappearance of the band at 1660 cm^{-1}, indicating that the band at 1660 cm^{-1} is an α, β unsaturated bond marker band. The change in carbonyl content during the wood decay process was also shown to be monitored using the Raman intensity of the carbonyl marker band. It was indicated that the NIR-FTR spectroscopic techniques were suitable analytical method for a rapid and nondestructive analysis of wood samples.

Keywords: Lignin; Carbonyl group; Nondestructive analysis; Fourier transform Raman spectroscopy; Near-infrared excitation laser

1. Introduction

A growing demand for wood and the preservation of natural resources strongly pertains not only to tree plantations but also to a development of novel biotechnologies. A lot of work has been directed towards the improvement of the planting stock via breeding, the selection, clonal propagation of elite genotypes, and production of genetically engineered trees as well as post-harvest improvement utilizing fungal or enzymatic treatments [1,2]. Lignocellulosic materials are abundant and renewable and their utilization is highly expected to be used as alternatives to fossil resources [3]. For chemical or biological treatment of wood, such as pulping process and alcohol

Abbreviations: DHP, dehydrogenated polymerizate (synthetic lignin); FTR, Fourier transform Raman; NIR, near-infrared.

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production from cellulose, not only the quantity but also the quality of lignin strongly affects the properties of the products and the cost efficiency. Rapid and nondestructive screening methods are of great importance, since huge numbers of progenies and chemically or biologically treated samples should be analyzed for the quality control and the establishment of new technology.

Vibrational studies such as infrared and UV–vis spectroscopies have been providing the structural information of plant materials. Among them, the Raman spectroscopic technique is advantageous since solid or powder samples can be measured directly. However, a conventional Raman spectroscopy using the excitation laser of the visible region causes a fluorescent emission interfering with the Raman measurement and causes sample damage due to the high energy of the visible laser. Using near-infrared (NIR) excitation laser dramatically reduced these problems [4]. Since the NIR laser does not greatly excite ν(O–H) in water, the moisture in the samples produces minimal effect on the spectra [5–7]. Therefore, the variation of samples from living tissue was enhanced, and no complicated procedures were required to obtain near-infrared Fourier transform Raman (NIR-FTR) spectra of lignocellulosic materials.

From these advantages, we utilized the NIR-FTR spectroscopic technique to determine the chemical structure of lignin. Previously, we have assigned the Raman marker bands for syringyl (S-) and guaiacyl (G-) nuclei, and proposed a rapid and nondestructive method to determine the S/G ratios in wood [8]. In this study, we examined the contributions of the carbonyl groups and α, β unsaturated bonds found in the side chain structure of lignin to the NIR-FTR spectra. NIR-FTR measurement of the lignin and a series of lignin model compounds, combined with the chemical treatments of lignin such as the reduction and reoxidation of samples, led us to assign the marker bands for the carbonyl groups and unsaturated bonds in native lignin. The quantitative results of carbonyl groups in lignin using the NIR-FTR were compared with those obtained from the conventional chemical methods, indicating that the Raman method is a rapid, easy-handling, and nondestructive technique to characterize lignin in a woody matrix.

2. Materials and methods

2.1. Wood samples

Japanese cedar (*Cryptomeria japonica* D. Don) was obtained from the experimental forest of Kyushu University. The sapwood was ground and fractionated using 100 and 42 mesh screens. The wood meal was extracted with ethanol–toluene (2:1 v/v) for 6 h in a Soxhlet apparatus [9]. Extractive-free wood meal (500 mg) was treated with a solution of $NaBH_4$ (100 mg) in 50% ethanol (50 ml) at 5 °C for 24 h with stirring [3]. After decantation, the wood meal was collected and suspended in water (50 ml) containing acetic acid (250 μl) and washed with water until the reaction mixture was neutral.

$NaBH_4$-reduced wood meal (300 mg) was reoxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (150 mg) in anhydrous dioxane (200 ml) under reflux for 72 h [10]. The color of wood meal after reoxidation was dark brown. The wood meal was then washed with ethanol and 1% NaOH.

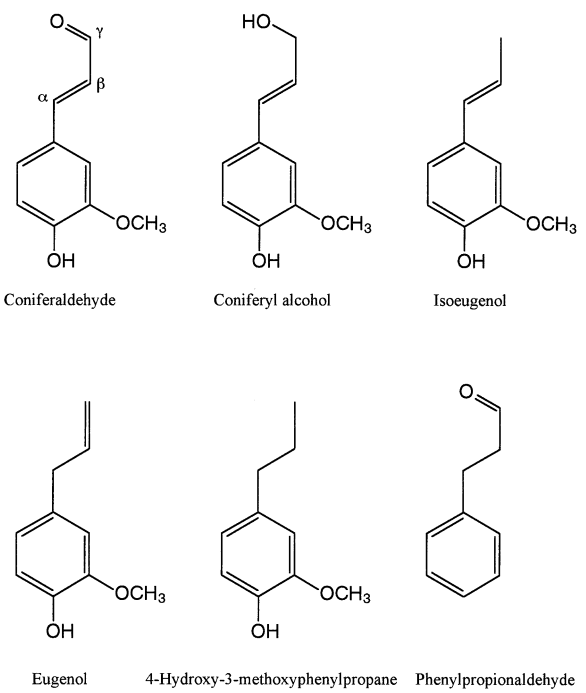
2.2. Isolation of lignin

Milled wood lignin (MWL) was isolated from the cedar wood meal according to the method of Björkman [11]. Wood meal was ground for 48 h in toluene using the vibrational ball mill. MWL was extracted with dioxane containing 10% water, precipitated with ether, and then centrifuged. The selective reduction of carbonyl groups in MWL with $NaBH_4$ was conducted in 50% ethanol for 12 h as described [12]. The benzyl hydroxyl group in $NaBH_4$-reduced MWL was reoxidized with DDQ in anhydrous dioxane for 20 h [13]. Hydrogenation was carried out in ethanol over palladium carbon (Pd; 5%) at room temperature to reduce either carbonyl groups or unsaturated bonds in alkyl side chain [14,15].

2.3. Model compounds

Coniferaldehyde was purchased from Aldrich. Coniferyl alcohol was prepared by reducing coniferaldehyde with $NaBH_4$ [16]. Coniferyl alcohol was identified using GCMS as a ditrimethylsilyl ether (m/z, M^+; 324). They were purified using flash chromatography (hexane/ethyl acetate, 3/1) before use. 4-Allyl-2-methoxyphenol (eugenol) and 2-methoxy-4-(1-propenyl)phenol (isoeugenol) were purchased from Tokyo Kasei and Wako Pure Chem., respectively. The ring-conjugated unsaturated bond in eugenol was hydrogenated in ethanol over palladium carbon (Pd 5%) to produce 4-hydroxy-3-methoxyphenylpropane. The hydrogenation reaction was carried out at room temperature. The chemical structures of model compounds were confirmed with MS spectrometry and shown in Scheme 1.

Chemically synthesized lignin (Dehydrogenated polymerizate; DHP) was prepared from either coniferyl alcohol (4-hydroxy-3-methoxybenzyl alcohol) or coniferaldehyde (4-hydroxy-3-methoxybenzaldehyde) using horseradish peroxidase (Sigma) and H_2O_2, forming Alc-DHP [17,18], and Ald-DHP [19,20], respectively. The yields were 60–70%. The colors of Alc-DHP and Ald-DHP were pale brown and reddish-brown, respectively.



Scheme 1. Chemical structures of lignin model compounds utilized in this study.

2.4. Instrumentation

NIR-FTR spectra were collected using a Perkin Elmer System 2000R spectrometer. The laser for excitation was diode Nd:YAG operating at 1064 nm. All spectra were obtained in the 180° scattering geometry. A quartz beamsplitter and InGaAs detector, averaging 200 scans with a resolution of 4 cm^{-1}, and a laser power of 200 mW were used to obtain FTR spectra in the range 3600–200 cm^{-1} or otherwise indicated. The curve-fitting (mixed Gaussian–Lorentzian) and peak area integration were performed using GRAMS/386TM software (Galactic Co.). IR spectra of wood meals and lignin model compounds were recorded using a Perkin Elmer System 2000 Fourier transform infrared (FTIR) spectrometer, with 64 or 100 scans and a resolution of 4 cm^{-1} using the KBr pellet method. FTIR spectra of wood meals were also obtained by the diffuse reflectance method. A GCMS analysis for lignin model compounds was performed using a JEOL Atomass-15A II system equipped with a fused silica column (NB-5; 30 m, GL Science) at 70 eV and 120–300 °C (8 °C min^{-1}). Samples were derivatized as previously described [21].

3. Results and discussion

Lignocellulosic materials found in wood and other plant cell walls are abundant and renewable, mainly consisting of cellulose, hemicellulose, and lignin [10,22]. Cellulose is the most abundant biomaterial but its utilization is retarded by lignin, which is one of the most recalcitrant biopolymers on Earth [23]. Thus, the removal of lignin is a very important issue not only for the pulping industry but also for utilizing carbohydrates as a starting material to produce sugars and alcohols in an industrial scale to build up the renewable and sustainable system (Green Industry) [2,24].

For the chemical and biological treatment of wood, structural information of lignin is essential. It is known that structural features of lignin, such as the ratio of syringyl nucleus to guaiacyl nucleus and the amount of ring conjugated carbonyl groups, exhibit a huge influence on the rate of delignification reaction and the quality of the final products in the pulping process [25]. It is also known that the ring-conjugated carbonyl groups in lignin cause the colorization of wood or the final products, when they are exposed to heat or UV light [26]. In the incipient stage of wood decay by fungi, the content of the carbonyl groups increase in proportion to the level of wood decay [27]. All these results suggest that ring-conjugated carbonyl groups of lignin exhibit a strong effect on the reactivity of lignin and the degree of deterioration. Although, several spectral methods to determine the total carbonyl content in lignin have been utilized, such as the absorption spectroscopy using hydroxylamine hydrochloride or sodium borohydride as a reducing reagent ($\Delta\varepsilon_r$; reduction difference spectra), the accuracy of these procedures is unpredictable because the model compounds used as standards for UV determination may not reflect the structure of native lignin [10]. Furthermore, the $\Delta\varepsilon_r$ method cannot be applied to the solid sample such as wood. Isolation of lignin involves tedious and time-consuming treatments, which may cause autooxidation to form a α-carbonyl group during the extraction and purification steps. Development of a rapid, nondestructive, and easy-handling procedure to directly determine the ring-conjugated carbonyl groups in wood would raise very unique and useful techniques to estimate the chemical features of lignin in a woody matrix.

3.1. NIR-FTR spectra of lignin model compounds

Fig. 1A and B shows the NIR-FTR spectra of coniferaldehyde and coniferyl alcohol, respectively. Either coniferaldehyde or coniferyl alcohol exhibited the bands at ~1600 and 1660 cm^{-1}. The bands at 1600 and 1585 cm^{-1} were assigned to ν(C=C) of an aromatic ring using the conventional Raman [28]. Besides those bands, only coniferaldehyde exhibited the extra band at 1620

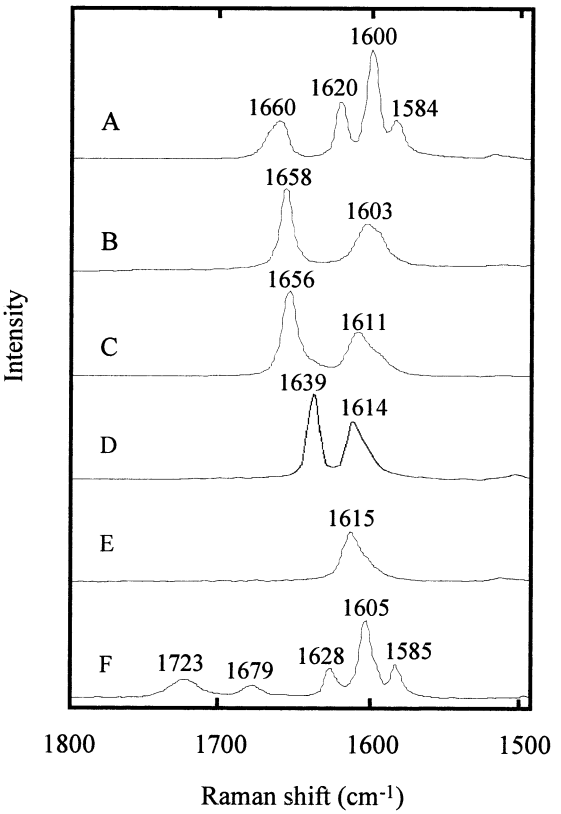


Fig. 1. NIR-FTR spectra of coniferaldehyde (A), coniferyl alcohol (B), isoeugenol (C), eugenol (D), 4-hydroxy-3-methoxyphenylpropane (E), and 3-phenylpropionaldehyde (F).

cm^{-1}. According to the structural features of two compounds (Scheme 1), this band was thought to be derived from the γ-carbonyl group. However, in a previous report, the band at 1661 cm^{-1} was assigned to the γ-carbonyl group using FTIR [29]. Then, those compounds were measured using FTIR. Fig. 2A shows the FTIR spectrum of coniferaldehyde, clearly showing the existence of the absorption bands at 1662 and 1618 cm^{-1}. On the other hand, the IR band at 1618 cm^{-1} was disappeared as the γ-carbonyl group was reduced to form coniferyl alcohol (Fig. 2B). These observations clearly indicated that the band at 1620 cm^{-1} is most likely derived from the stretching of the carbonyl group and that the band at ~1660 cm^{-1} could be assigned to the ring-conjugated α, β unsaturated bond.

To further characterize the contribution of the ring-conjugated α, β unsaturated bond to the NIR-FTR spectra, the NIR-FTR spectrum of isoeugenol was measured. It showed a strong band at 1656 cm^{-1} with the aromatic band at 1611 cm^{-1}, but no band was observed in 1620 cm^{-1} region (Fig. 1C). In addition, no Raman band was detected in the region of 1655–1660 cm^{-1} in the NIR-FTR spectra of either eugenol or 4-hydroxy-3-methoxyphenylpropane, containing no ring-conjugated α, β unsaturated bond (Fig. 1D and E). These results supported that the Raman band found at 1655–1660 cm^{-1} could be assigned to the ring-conjugated α, β unsaturated bond. Phenylpropionaldehyde exhibited a band at 1628 cm^{-1}, supporting that the Raman band at ~1620 cm^{-1} is the marker band for the carbonyl stretching (Fig. 1F). It is also suggested from the FTR spectra of model compounds (Fig. 1) that the Raman shift of the carbonyl marker band is dependent on whether the carbonyl is conjugated to either an α, β unsaturated bond or to an aromatic ring.

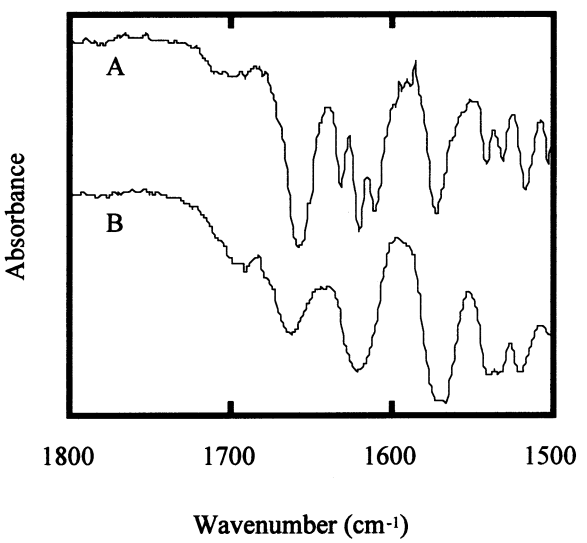


Fig. 2. FTIR spectra of coniferaldehyde (A) and coniferyl alcohol (B).

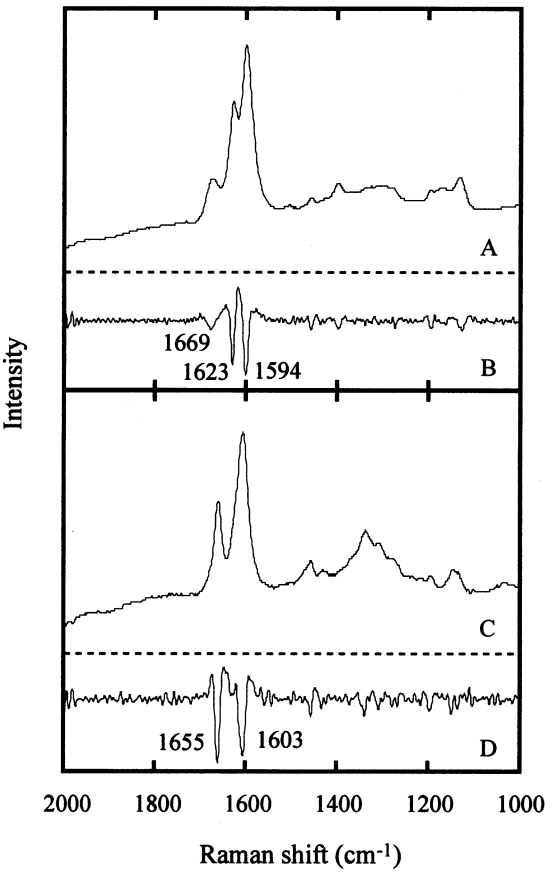


Fig. 3. NIR-FTR spectra of Ald-DHP (A) and Alc-DHP (C), and their second derivative (B and D).

3.2. NIR-FTR spectra of polymeric lignin

Lignin in a woody matrix is an amorphous aromatic polymer formed from phenylpropanoid precursors, such as coniferyl and sinapyl alcohols, via random radical coupling reactions [22]. Since the chemical analysis of lignin requires tedious and time-consuming steps, we initiated the utilization of NIR-FTR spectroscopic techniques. Synthetic polymer lignin samples (DHPs) were prepared. Fig. 3A and C show the NIR-FTR spectra of DHPs synthesized from coniferaldehyde (Ald-DHP) and coniferyl alcohol (Alc-DHP), respectively. Only the NIR-FTR spectrum of Ald-DHP exhibited the band at 1623 cm^{-1}, supporting that this Raman band was derived from the carbonyl group. These observations cor-

respond well with a previous report, which indicated that the carbonyl groups are much more abundant in Ald-DHP than those in Alc-DHP [30]. The band at 1625 cm^{-1} was also found in the NIR-FTR spectra of MWL and extractive-free wood meal, albeit with weaker intensity (data not shown).

3.3. Assignment of the carbonyl band in lignin

To further characterize the Raman bands derived from lignin found at the 1500–1700 cm^{-1} region, a computer-fit analysis was applied to the Raman data. The precise position and number of the Raman bands were estimated from the second derivative of the spectra. These values are required for the spectral curve-fit (peak component) analysis to determine the detailed assignment of the Raman bands [31]. The second derivatives of the NIR-FTR spectra of Ald-DHP and Alc-DHP clearly show the band position and number existing in 1500–1700 cm^{-1} region (Fig. 3B and D). Then, the curve-fit analysis was performed, showing a very good correlation between experimental and computer-calculated curves (curves *a* and *b* in Fig. 4). Using these analytical methods, the effect of the reduction of carbonyl groups using $NaBH_4$ on DHP was examined. $NaBH_4$ has been reported to reduce carbonyl groups in lignin structure to corresponding hydroxyl groups but to leave the ethylene group intact [12]. Fig. 4A and B show the NIR-FTR spectra of Ald-DHP and its reduced sample. These spectra were subjected to a peak component analysis. The reduction of all carbonyl groups caused complete disappearance of the bands at 1623 cm^{-1}. The Raman characteristics of $NaBH_4$-reduced DHP were almost identical to Alc-DHP (Fig. 3C and Fig. 4B). Then, reoxidation of $NaBH_4$-reduced Ald-DHP using DDQ was carried out. DDQ has been known to oxidize the benzyl hydroxyl group to the corresponding carbonyl group [13,16]. In the spectrum of DDQ-reoxidized DHP, a strong band at 1622 cm^{-1} was again observed, which was assigned to the carbonyl marker band as described above. The aromatic C=C stretching band was seen at 1596 cm^{-1}, which was observed at 1594 cm^{-1} in Ald-DHP (Fig. 4C). Although the Raman characteristics of the reoxidized DHP were very similar to those of the original Ald-DHP, there were some differences. The intensity ratio of the peak at 1622 cm^{-1} to the aromatic band at 1596 cm^{-1} seen in the spectrum of reoxidized DHP was larger than that of the peak at 1623–1594 cm^{-1} seen in Ald-DHP. Since Ald-DHP contains the γ-carbonyl group and the reoxidized DHP should contain the α-carbonyl group, we tentatively concluded that the Raman shift of either ring-conjugated α-carbonyl and γ-carbonyl groups appeared at the same region of 1620–1625

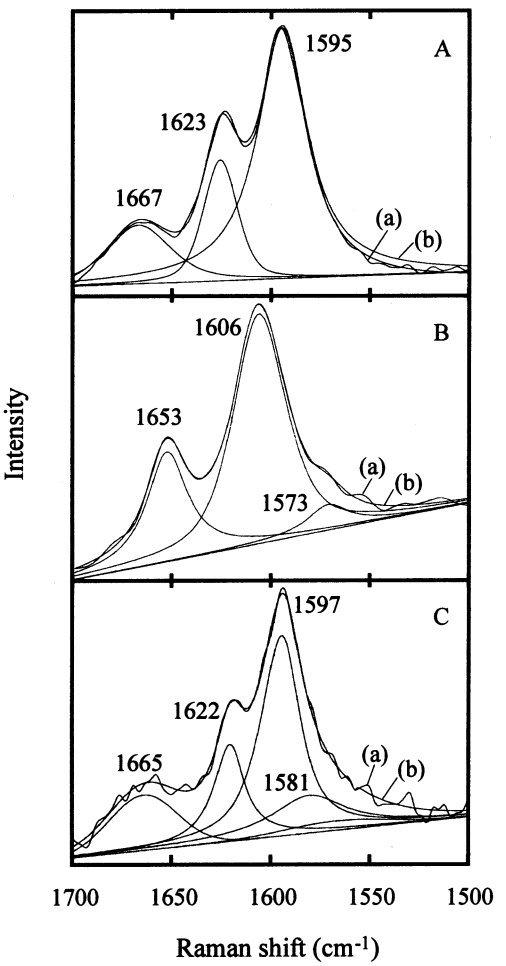


Fig. 4. NIR-FTR spectra of Ald-DHP (A), A reduced with $NaBH_4$ (B), and B reoxidized with DDQ (C). Line *a* indicates the experimental spectrum and line *b* indicates the calculated spectrum from curve-fit data.

cm^{-1} and that the ring-conjugated α, β unsaturated bond is detected at 1660 cm^{-1}, which was almost unchanged during $NaBH_4$-reduction and DDQ-reoxidation processes. It is also suggested that the intensity of the carbonyl marker bands depends on how strongly they conjugated to other C=C bonds such as an aromatic ring and α, β unsaturated bond. In addition, any bands derived from DDQ did not disturb the measurement of the NIR-FTR spectra of DHPs (data not shown).

The effect of $NaBH_4$-reduction and DDQ-reoxidation treatments on native lignin was also examined. In the NIR-FTR spectra of MWL and wood meal, the band at 1620–1625 cm^{-1} disappeared upon $NaBH_4$ reduction, and appeared again upon reoxidation with DDQ (data not shown). From these results, we concluded that the ring conjugated carbonyl groups are detected at ~1625 cm^{-1} in the NIR-FTR spectra.

3.4. Assignment of the ring-conjugated α, β unsaturated bond

In the early stage of this study, we found that the band at 1660 cm^{-1}, once assigned to carbonyl vibration from FTIR study [29], should be assigned to the α, β unsaturated bond in NIR-FTR spectroscopy using model compounds. Therefore, it ought to further characterize the α, β unsaturated bond in lignin using the FTR technique. The effect of the reduction of the α, β unsaturated bond on the FTR spectra was examined using Pd:C-catalyzed hydrogenation. Through this reduction reaction, both the unsaturated bonds and the carbonyl groups in the lignin side chains are completely reduced, but leave the aromatic ring unchanged. In the NIR-FTR spectra of lignin model compounds, the α, β unsaturated bond was detected at 1656–1660 cm^{-1} (Fig. 1). The band at ~1660 cm^{-1} was also observed in the NIR-FTR spectra of Alc-DHP, Ald-DHP, MWL, and wood meal. Fig. 5A shows the NIR-FTR spectrum of $NaBH_4$-reduced MWL, where this band was still observed even though the band at 1620 cm^{-1} completely disappeared. However, upon the hydrogenation of the sample, the band at 1660 cm^{-1} disappeared (Fig. 5B). The complete disappearance of this band was also observed against the hydrogenation of Ald-DHP, suggesting that the peak at ~1660 cm^{-1} is assigned to the ring-conjugated α, β unsaturated bond in lignin.

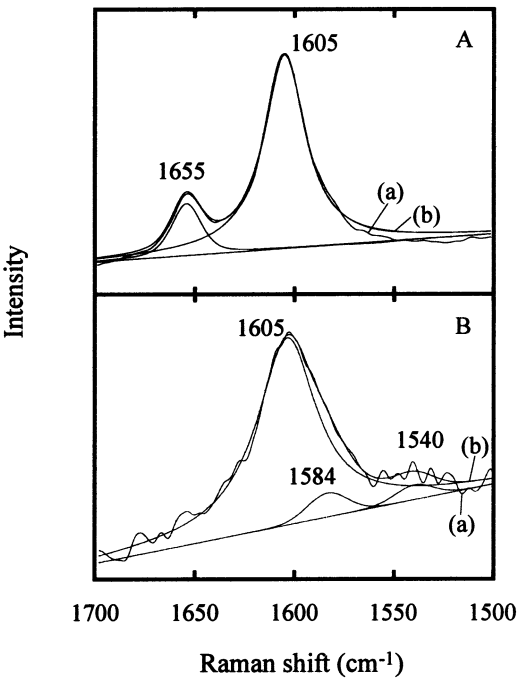


Fig. 5. NIR-FTR spectra of MWL after reduction with $NaBH_4$ (A), and A further hydrogenated (B). Line *a* indicates the experimental spectrum and line *b* indicates the calculated spectrum from curve-fit data.

3.5. Monitoring the change in ring conjugated carbonyl content

To apply the NIR-FTR spectroscopic techniques for monitoring the carbonyl group contents in lignin, the Raman intensity ratio of 1625–1600 cm^{-1} was compared with the results obtained from the $\Delta\varepsilon_r$ method (Table 1). The aromatic marker band at 1600 cm^{-1} was utilized as an internal standard to minimize the deviation of the Raman intensities among the samples, since both aromatic and carbonyl marker bands were derived from lignin. The conventional method revealed that the increase of the carbonyl groups in MWL and Alc-DHP when treated with DDQ. The ratio of the Raman bands for the carbonyl

Table 1
Contents of the ring conjugated carbonyl groups determined by the conventional $\Delta\varepsilon_r$ method and FTR spectroscopic method

Lignin samples	$\Delta\varepsilon_r$ method (Number/100 C_9 units)	FTR method (1625/1600 cm^{-1})
MWL		
No treatment	13.2	1.50
DDQ-oxidation	17.4	1.85
Alc-DHP		
No treatment	0	0
DDQ-oxidation	4.2	1.31

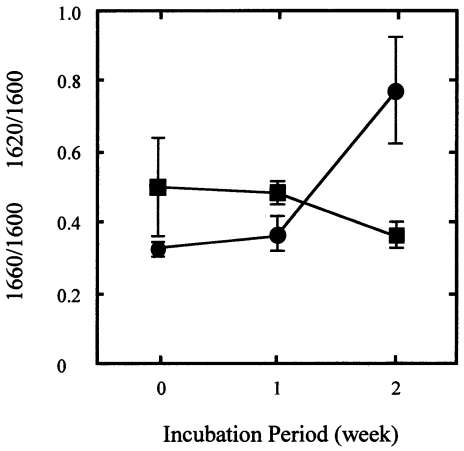


Fig. 6. Time course of the change in Raman intensity ratio during wood decay process. 1660/1600 cm^{-1} indicates the decrease of α, β unsaturated bond (■) and 1620/1600 cm^{-1} indicates the increase of carbonyl contents (●) in wood sample (*C. japonica*) during fungal decay by *C. versicolor*.

marker (1625 cm^{-1}) and the aromatic marker (1600 cm^{-1}) also increased as the samples were DDQ-oxidized. Since the Raman carbonyl marker band counts both α- and γ-carbonyl groups, further consideration of lignin structure would be still required for quantitative determination of the ring-conjugated carbonyl group. However, it was also indicated that the change in relative contents of the ring-conjugated carbonyl groups in lignin could be monitored by measuring the intensity ratio of carbonyl marker band and the aromatic marker band (Table 1).

Upon fungal treatment of wood, it is known that the content of the ring conjugated carbonyl groups increases, and the content of the ring conjugated α, β unsaturated bond decreases [23]. Therefore, for the bands at 1620 and 1660 cm^{-1}, it would be useful to know the degree of wood decay or deterioration. Fig. 6 shows the time course of the change in Raman intensity ratio of 1620/1600 and 1660/1600 cm^{-1}, indicating the increase of carbonyl contents and the decrease of unsaturated bonds in the early stage of fungal decay of wood. During the early stage of wood decay, the content of aromatic moiety could be assumed to be unchanged.

4. Conclusion

From the Raman study of a series of lignin model compounds, DHPs, MWL, and wood meal, it was indicated that the bands at 1620 and 1660 cm^{-1} are assigned to the marker bands for carbonyl groups and for the α, β unsaturated bond, respectively. Using these marker bands, the change in the relative contents of the carbonyl groups and α, β unsaturated bonds can be monitored. It was strongly suggested that NIR-FTR spectroscopic technique would be useful for the rapid and nondestructive determination of the ring-conjugated carbonyl groups of lignin in a woody matrix.

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