

# Determination of ethylenic residues in wood and TMP of spruce by FT-Raman spectroscopy

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## Abstract

A method based on FT-Raman spectroscopy is proposed for determining in situ concentrations of ethylenic residues in softwood lignin. Raman contributions at 1133 and 1654  $\text{cm}^{-1}$ , representing coniferaldehyde and coniferyl alcohol structures, respectively, were used in quantifying these units in spruce wood with subsequent conversion to concentrations in lignin. For coniferaldehyde units, the intensity of the 1133  $\text{cm}^{-1}$  peak was measured in the difference spectrum obtained by subtracting the bleached-wood spectrum from that of the unbleached. In the case of coniferyl alcohol residues, the intensity of the 1654  $\text{cm}^{-1}$  band was calculated from the spectrum of extensively bleached wood. The concentrations of coniferaldehyde and coniferyl alcohol units in spruce lignin were found to be 3.8% and 3.4%, respectively, and were in good agreement with values determined by conventional techniques. This quantification of the ethylenic residues was based on the Raman intensities of 1% coniferaldehyde and 1% coniferyl alcohol in bleached softwood kraft pulp. Initially, as background for this work, a number of suitable lignin model compounds and a softwood lignin model polymer (G-DHP) were used to calibrate the Raman method and demonstrate that the Raman technique was well suited for quantification of ethylenic structures. Experimental results demonstrated that thermomechanical pulping reduced the concentrations of coniferaldehyde and coniferyl alcohol residues in comparison to wood by 28% and 24%, respectively.

**Keywords:** coniferaldehyde; coniferyl alcohol; DHP; ethylenic residues; lignin; quantification; Raman; thermomechanical pulp; wood.

## Introduction

In softwood lignin, ethylenic units are present at low concentrations mainly as coniferaldehyde and coniferyl alcohol residues. Information on the concentrations is important not only in the studies of lignin structure and chemistry (e.g., wild-type vs. transgenic plant lignin and bleaching chemistry) but also in applications where these structures play a significant role in material properties

(e.g., mechanical pulp brightness). Although there are established procedures for determining concentration of ethylenic groups, most require that lignin be isolated first (Aulin-Erdtman and Hegbom 1958; Marton and Adler 1961; Dence 1992; Liitiä et al. 2003; Capanema et al. 2004). Both methods based on UV (Aulin-Erdtman and Hegbom 1958; Marton and Adler 1961) and  $^{13}\text{C}$  NMR spectroscopy (Liitiä et al. 2003; Capanema et al. 2004) fall into this category. The degradative methods, such as thioacidolysis (Lapierre et al. 1986) and nitrobenzene oxidation (Ito et al. 1999), cannot be considered quantitative because the reactive ethylenic components might be partially lost during the degradation procedure (Ito et al. 1999). Dence (1992) reviewed the traditionally available methods for the determination of ethylenic residues.

The structure of milled-wood lignin (MWL) has been extensively investigated. However, MWL is not representative of the whole lignin of a tree, because the yields are low and lignins derived from different morphological regions may be over- or under-represented (Maurer and Fengel 1992; Terashima et al. 1992). Moreover, the isolation steps – mainly the milling – involved modify lignin chemically to some degree (Ikeda et al. 2002; Guerra et al. 2006). In the present work, a new approach based on FT-Raman spectroscopy is proposed that permits in situ quantification of the ethylenic structures in wood.

Raman spectroscopy is well suited for in situ analysis of composite lignocellulosic materials without isolating their components. The technique is also non-destructive, meaning that the sample can be analyzed further by other methods. A number of FT-Raman applications to the field of lignocellulosics have been reported (Evans 1991; Scheepers et al. 1993; Agarwal and Ralph 1997; Yamauchi et al. 1997; Holmgren et al. 1999; Niemelä et al. 1999; Ona et al. 1999; Yang et al. 1999; Proniewicz et al. 2002; Agarwal et al. 2003, 2005; Edwards et al. 2003; Yamauchi and Kurimoto 2003; Agarwal and Landucci 2004; Vester et al. 2004; Yamauchi et al. 2005). Moreover, Raman spectroscopy has a high sensitivity to detecting aromatic-ring conjugated groups (Schmid and Brosa 1971; Schmid and Topsom 1981; Agarwal and Atalla 2000; Agarwal et al. 2005), which in lignin are present in small concentrations. Accordingly, the technique seems to be very well suited to quantifying such groups. Ethylenic groups have been easily detected in the Raman spectra of lignin models and lignocellulosics (Agarwal and Atalla 1993; Agarwal et al. 1995, 2005; Agarwal 1999).

Raman spectroscopy is a quantitative method as long as the variations associated with Raman instrumentation and sampling issues are correctly accounted for. One way of accomplishing this is by using an internal standard band of the solvent/sampling medium. To demonstrate this capability of Raman, a number of simple and polymeric lignin model compounds (guaiacyl-DHP, G-

DHP), were analyzed quantitatively. Suitable Raman bands were selected for quantification, the effect of derivatization on Raman scattering coefficients was evaluated, calibration lines for models were developed, and the overall approach was validated with a DHP model. To compare Raman and NMR methods, ethylenic groups in coniferyl alcohol diacetate and G-DHP were measured.

## Materials and methods

### Lignin models, G-DHP, and MWL

Coniferaldehyde (**I**) and coniferyl alcohol (**II**) (purity, 98% and >97%, respectively) were purchased from Sigma-Aldrich. G-DHP (**III**) was a gift from Professor Noritsugu Terashima. The DHP was produced from coniferin by means of the enzymes  $\beta$ -glucosidase and peroxidase (Terashima et al. 1995). The MWL isolation is reported elsewhere (Agarwal and Ralph 1997). Derivatized lignin models, methoxy coniferaldehyde (**IV**), methoxy coniferyl alcohol (**V**) and coniferyl alcohol diacetate (**VI**), were prepared in our laboratory.

**Methoxy coniferaldehyde (IV) I** (100 mg, 0.56 mmol) was dissolved in 10 ml of acetone. Methyl iodide (239 mg, 1.68 mmol, 105  $\mu$ l) was added along with freshly powdered anhydrous potassium carbonate (233 mg, 1.68 mmol). The mixture was magnetically stirred overnight and checked by thin layer chromatography (TLC). The mixture was filtered and the residue washed with acetone. The combined acetone solutions were dried. The reaction mixture was taken up in chloroform and extracted three times with water. The chloroform layer was dried over anhydrous magnesium sulfate. The final product was purified by preparative TLC on Whatman PLK5F 1000  $\mu$ m silica gel plates. The eluent was 1 ml methanol in 250 ml of methylene chloride, one development.

**Methoxy coniferyl alcohol (V)** Starting with **II**, **V** was prepared in a manner similar to that of compound **IV**.

**Coniferyl alcohol diacetate (VI) II** (100 mg) was dissolved in 0.5 ml of pyridine in a 15-ml round-bottom flask. Acetic anhydride (0.5 ml) was added, the flask was closed, and the solution allowed to react for 30 min. Toluene (7 ml) was added and the mixture was roto-evaporated to dryness. The toluene co-evaporation was repeated twice more and was followed by three co-evaporations with acetone until constant weight was achieved.

For generating calibration curves, different concentrations of **I** (4, 2, 1, 0.5, and 0.25%) and **II** (4, 2, 1, 0.5, and 0.25%) were prepared in methanol and were analyzed using FT-Raman spectroscopy. Additionally, **II** was quantified in bleached softwood kraft pulp (BSKP, National Institute of Standards and Technology reference material 8495, Gaithersburg, MD, USA) by FT-Raman. For the BSKP quantification, pellets were made according to the procedure described below. Similarly, to compare quantification capability of Raman with  $^{13}\text{C}$  NMR, not only several concentrations of **VI** were prepared in  $\text{CDCl}_3$ , but also the coniferyl alcohol residues in **III** were quantified in dioxane solutions of **III** [1.21% or 0.0654 M (C9 unit weight 185)]. For the latter, compounds **II** (0.023 M in dioxane) and **I** (at low concentration in dioxane) were submitted to quantitative spectroscopy. In such quantification work, either the solvent band (1015, 1035, and 2252  $\text{cm}^{-1}$ , respectively, for dioxane, methanol, and  $\text{CDCl}_3$ ), or in the case of BSKP samples, 1096  $\text{cm}^{-1}$  cellulose band was used as an internal standard. Solutions were sampled in capped NMR tubes.

## NMR spectroscopy

The sample **III** was run with standard Bruker pulse sequences on a Bruker DPX-250 (62.9 MHz  $^{13}\text{C}$ ) spectrometer (Bruker Instruments, Inc., Billerica, MA, USA) fitted with a quadrupole 5-mm Z-gradient coil probe. The sample (~100 mg) was dissolved in 400  $\mu$ l of  $\text{DMSO-}d_6$ . The central solvent peak served as the internal reference: DMSO at  $\delta_{\text{H}}$  2.50,  $\delta_{\text{C}}$  39.5 ppm. The quantitative  $^{13}\text{C}$  spectral experiment was acquired with an inverse-gated sequence, a 15-s relaxation delay, 90° pulse, and 9000 scans. The spectrum was integrated between 130 and 127.5 ppm for the two unsaturated methine carbons and the methoxyl peak centered at 56 ppm. The methoxyl peak was set to 100 (one methoxyl per  $\text{C}_9$  unit) and the area under the unsaturated methines was divided by two to represent one side chain. The integration procedure was carried out four times based on a line broadening of 0, 2 (twice), and 4 Hz: an average value of  $25.8 \pm 0.5$  unsaturated side chains per 100  $\text{C}_9$  units was obtained.

## Pellet samples for Raman

The BSKP was Wiley-milled to pass a 40-mesh screen. This pulp, 324.9 mg (oven-dried, O.D.), was placed in a vial and 2-ml methanol solution containing 36.1 mg of **II** was added. After 10 min, the pulp was subjected to vacuum until a constant weight was obtained at 361 mg. An additional 2 ml of methanol was added, the mixture was agitated, and the mixture was brought to a constant weight under vacuum for a final weight of 361 mg. This BSKP sample, therefore, had a **II** concentration of 10%. The remaining concentrations of **II** in BSKP (5, 2.5, 1.25, and 0.625%) were prepared by diluting the 10% pulp mixture with the appropriate amount of the control pulp in a small vial and then subsequently tumbling the mixture. After this, the mixture was wetted with methanol and once the solvent evaporated, the mixture was tumbled again. Pellets for Raman analysis were made with an infrared KBr press. Wood and thermomechanical pulp (TMP) samples were also analyzed in the pellet form. In a pellet, approximately 0.2 g of wood or TMP was included.

Likewise, samples of **I** (0.93%) and **II** (0.79%) in BSKP were prepared by mixing 4.7 mg of **I** with 500 mg BSKP and 4 mg **II** with 500 mg BSKP. Several pellets from each sample were produced and analyzed. The average intensities and standard deviations associated with selected Raman bands were calculated and are reported.

## Wood and wood-bleaching

Spruce-wood chips (an equal mixture of black and white spruce, *Picea mariana* and *P. glauca*, respectively) were Wiley-milled to pass a 40-mesh screen. The milled wood was extracted successively with acetone/water (9:1; v/v) and toluene/ethanol (2:1; v/v). Small amounts of wood samples were bleached with  $\text{NaBH}_4$  and alkaline peroxide by the following procedures.

Bleaching of milled spruce wood (2% consistency) with 0.5 M  $\text{NaBH}_4$  (reducing agent) was carried out at room temperature for 2 h in aqueous medium. Aldehyde and ketone groups in wood are reduced by  $\text{NaBH}_4$ . Raman analysis of twice  $\text{NaBH}_4$ -treated samples indicated no further bleaching effect on the bands of interest, and therefore borohydride treatment was considered as complete over the 2-h duration of the treatment. The excess borohydride was destroyed by slowly acidifying the reaction suspension to pH 6 with concentrated HCl. The borohydride-brightened wood was isolated after filtering the suspension through a coarse fritted glass funnel and washing the wood residue with excess deionized water.

The spruce wood was oxidatively bleached using alkaline hydrogen peroxide. The bleaching conditions for wood on O.D.

weight basis were 5% H<sub>2</sub>O<sub>2</sub>, 5.3% total alkali, 0.1% MgSO<sub>4</sub>, and 4% Na<sub>2</sub>SiO<sub>3</sub>. The initial pH just after mixing chemicals with pulp was 11.5, and bleaching was performed at 10% consistency. Milled wood and the bleaching solution were put in a reclosable plastic bag and mixed by kneading. The bag was kept in a 70°C water bath for 3 h. The wood was periodically remixed by kneading to ensure uniform distribution of bleaching chemicals. After 3 h, the wood was filtered and washed first with deionized water and then with dilute sodium bisulfite solution.

### Thermomechanical pulp

Spruce TMP, produced from a mixture of 50:50 white and black spruce, was the same as investigated and described earlier (Agarwal and Landucci 2004).

### Raman spectroscopy

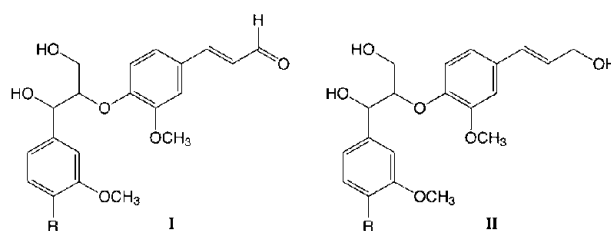
The FT-Raman spectra were obtained by a Bruker RFS-100 spectrometer (Bruker Optics Inc., Billerica, MA, USA) equipped with a 1-W 1064-nm Nd:YAG diode laser. Samples were analyzed in the 180° scattering geometry and the power at the sample was 600 mW. For each spectrum, 1024 scans were accumulated using the double-sided forward-backward scanning mode. Solutions were sampled in 5-mm capped NMR tubes, and BSKP, wood, and TMP samples were studied as pressed pellets. Two sequential spectra of each sample were obtained to ensure the laser was not modifying the sample in any way. The chosen internal standard bands (in solution and solid sample spectra) were used to calculate relative peak heights to compare spectra between samples. Use of the internal standards took care of the variations caused by the instrument and sampling issues. Instrument and spectral manipulations: OPUS software provided by the Bruker instrument company.

All band heights were measured by the sloping baseline method. The measurements at 1133 and 1654 cm<sup>-1</sup> represented, respectively, coniferaldehyde and coniferyl alcohol groups. In solutions, the relative band intensity was calculated by dividing the peak height measurement by appropriate solvent band intensity. For the **I** and **II** in BSKP samples, several samples of a given composition were analyzed and the measured peak height was converted to relative intensity by dividing it by the cellulose band intensity at 1096 cm<sup>-1</sup> ( $I_{1133}/I_{1096}$  and  $I_{1654}/I_{1096}$ ). Average relative intensity and standard deviation were calculated. For convenience, the relative intensity data were converted to 1% sample weight basis for these BSKP samples by multiplying each intensity ratio by the factor representing the ratio of 1% to actual concentration in BSKP [1.075 (1/0.93) and 1.266 (1/0.79) for **I** and **II**, respectively].

The BSKP samples had 99% holocellulose (spruce wood around 68%, Pettersen 1984), indicating a very low lignin content. Therefore, the relative intensity data obtained from the wood samples were divided by 1.46. These "corrected quotients" ( $I_{1133}/I_{1096}$  and  $I_{1654}/I_{1096}$ ) were used in the quantification of the ethylenic residues by comparing the data to the Raman intensities of 1% **I** and 1% **II** in BSKP values. When applying this method to different woods and pulps, other factor than 1.46 will be needed due to their different holocellulose contents.

## Results and discussion

Figure 1 shows coniferaldehyde and coniferyl alcohol end groups in guaiacyl type lignin structures, which are representative for ring-conjugated ethylenic structures. As a rule, these ethylenic residues are attached to β-O-4 linkages via ether bonds in the phenolic position. Link-



**Figure 1** Lignin β-O-4 dimer structural units with coniferaldehyde (**I**) and coniferyl alcohol (**II**) as end groups.

ing through C5 is also possible, but this possibility is more typical for DHPs than for native lignins. Raman bands associated with ethylenic units of coniferaldehyde and coniferyl alcohol in lignin-containing materials have been identified (Agarwal and Atalla 1993; Agarwal et al. 1995; Agarwal 1999; Agarwal and Atalla 2000). These data are summarized in Table 1.

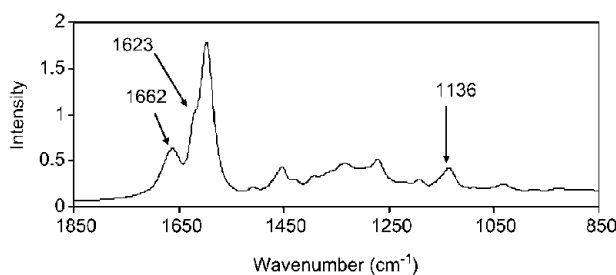
The two bands listed in Table 1 at 1660 and 1654 cm<sup>-1</sup> associated with coniferaldehyde and coniferyl alcohol, respectively, overlap because of neighboring band positions. Moreover, earlier work (Agarwal 1996) has shown that the coniferaldehyde's C=O stretch mode at 1660 cm<sup>-1</sup> is subject to shift depending upon how strongly the C=O is hydrogen-bonded. In that study, the band position varied between 1685 cm<sup>-1</sup> (in CCl<sub>4</sub>) and 1657 cm<sup>-1</sup> (in cellulose). On the contrary, the C=C stretch in coniferyl alcohol (1654 cm<sup>-1</sup> band) is expected to vary only little, because this mode is likely to be affected by the intermolecular interactions in a limited way (Agarwal 1996).

An FT-Raman spectrum of black spruce MWL is presented in Figure 2 (Agarwal and Ralph 1997). In the spectrum, the bands belonging to the ethylenic units are

**Table 1** Useful Raman frequencies of coniferaldehyde and coniferyl alcohol in cellulose (assignment in parentheses).

Coniferaldehyde	Coniferyl alcohol
1136 cm <sup>-1</sup> (C-H bending)	–
1599 cm <sup>-1</sup> (Aromatic ring mode)	1608 cm <sup>-1</sup> (Aromatic ring mode)
1623 cm <sup>-1</sup> (C=C stretch)	–
1660 cm <sup>-1</sup> (C=O stretch)	1654 cm <sup>-1</sup> (C=C stretch)

Small shifts in the band positions are possible, due to overlapping band profiles and/or presence of intermolecular interactions.



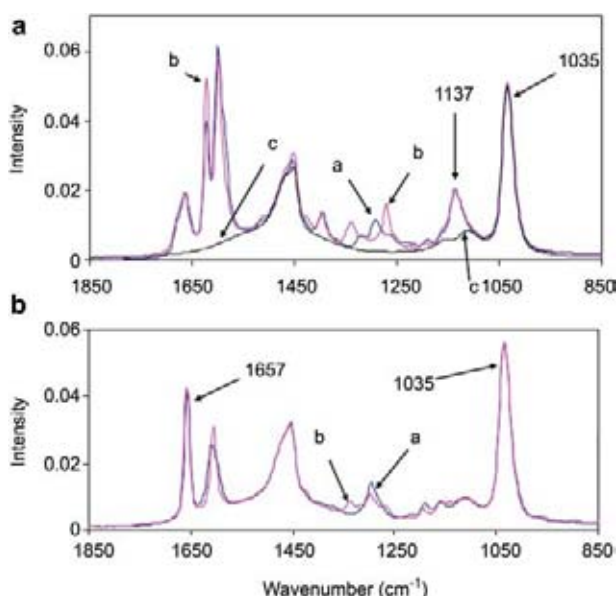
**Figure 2** The FT-Raman spectrum of black spruce MWL in the 1850–850 cm<sup>-1</sup> region. The band at 1136 cm<sup>-1</sup> is caused by coniferaldehyde groups, and the other two (1662 and 1623 cm<sup>-1</sup>) have underlying contributions from coniferyl alcohol and other chromophores in lignin.

annotated at 1136, 1623, and 1662  $\text{cm}^{-1}$ . Except for the band position at 1136  $\text{cm}^{-1}$ , where the contribution is entirely due to the coniferaldehyde units, the other positions (1623 and 1662  $\text{cm}^{-1}$ ) have contributions from not only coniferyl alcohol and coniferaldehyde (Table 1) but also from other chromophore structures in lignin (Agarwal and McSweeney 1997; Agarwal and Landucci 2004). For example, when spectra of unbleached and bleached TMPs were compared in this region, it was observed that bleaching resulted in the removal of contributions in addition to that of coniferaldehyde. From this study, it is likely that quinonoid type chromophores contribute to this region.

### Effect of derivatization on band intensity

The effect of the methylation of the *p*-phenolic OH group in coniferaldehyde and coniferyl alcohol on the Raman intensity of selected bands is of scientific interest. The bands at 1133 and 1654  $\text{cm}^{-1}$  were selected for this purpose as the quantification studies were also performed based on these bands. Note that these band positions are prone to shift slightly due to intermolecular interactions (see Table 1).

Raman intensities around 1137  $\text{cm}^{-1}$  for coniferaldehyde (I) and its methylated form (IV) (both 0.1122 M in methanol) can be compared (Figure 3a) in the normalized spectra. For the normalization, the 1035  $\text{cm}^{-1}$  band of methanol was chosen because this band does not interfere with the bands of I. The intensity of band 1137  $\text{cm}^{-1}$  in spectra (a, I) and (b, IV) is similar. The methanol spectrum, designated with (c), is clearly visible between 1050 and 1150  $\text{cm}^{-1}$  and 1500 and 1700  $\text{cm}^{-1}$ . Although the intensities of the bands at 1623 and 1600  $\text{cm}^{-1}$  showed some variation, the 1137  $\text{cm}^{-1}$  intensity was the



**Figure 3** (a) FT-Raman spectra of (a) 0.1122 M coniferaldehyde (I) in methanol, (b) 0.1122 M methoxy coniferaldehyde (IV) in methanol, and (c) pure methanol in NMR tubes in the region 1850–850  $\text{cm}^{-1}$ . (b) Spectra of (a) 0.4276 M coniferyl alcohol (II) in methanol and (b) 0.4276 M methoxy coniferyl alcohol (V) in methanol, in NMR tubes in the region 1850–850  $\text{cm}^{-1}$ . Methanol band at 1035  $\text{cm}^{-1}$  was used to normalize the spectra.

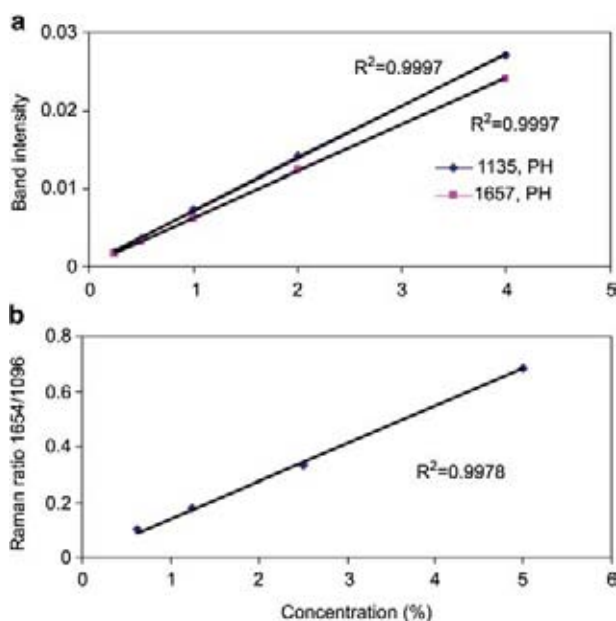
same between the derivatized and non-derivatized models. It is obvious that methylation at the *p*-position did not lead to any significant change in the scattering coefficient of this Raman band. Therefore, the 1137  $\text{cm}^{-1}$  band is suitable for quantification both for etherified and non-etherified coniferaldehydes.

The same is true for the band intensities around 1657  $\text{cm}^{-1}$  (small shifts are also possible here, see Table 1) for coniferyl alcohol (II) and its methoxy derivative (V). If the intensities of this band were compared on a molar basis (0.4276 M), once again, no significant intensity change was observed upon the introduction of the methoxy group (Figure 3b). Accordingly, this band is useful for quantification both for etherified and non-etherified coniferyl alcohol units.

### Calibration curves based on models

**Coniferaldehyde (I) and coniferyl alcohol (II)** Calibration curves for I and II were generated in the concentration range between 0.25% and 4% (Figure 4a). Also here, the methanol band at 1035  $\text{cm}^{-1}$  was selected for internal standard to calculate band heights. As can be noted from Figure 4a, in both cases, excellent correlations between Raman intensity and actual concentrations were obtained. This demonstrates that Raman is suited for quantitative applications, and the variations associated with the technique can be avoided by the internal standard method.

To evaluate the effect of wood matrix, a similar analysis was carried out with coniferyl alcohol (II) in BSKP. The relative intensity of the 1654  $\text{cm}^{-1}$  band (related to 1096  $\text{cm}^{-1}$  band of BSKP as an internal standard) was plotted against the actual concentrations (Figure 4b). A good correlation can be confirmed.



**Figure 4** (a) Calibration lines for quantitation of coniferaldehyde (I) (1135  $\text{cm}^{-1}$  peak height, PH) and coniferyl alcohol (II) (1657  $\text{cm}^{-1}$  band peak height) in methanol by FT-Raman. In both cases, Raman intensity data correlated very well with actual concentrations. (b) Quantification of coniferyl alcohol (II) in bleached-softwood kraft pulp by FT-Raman (1654  $\text{cm}^{-1}$  band).

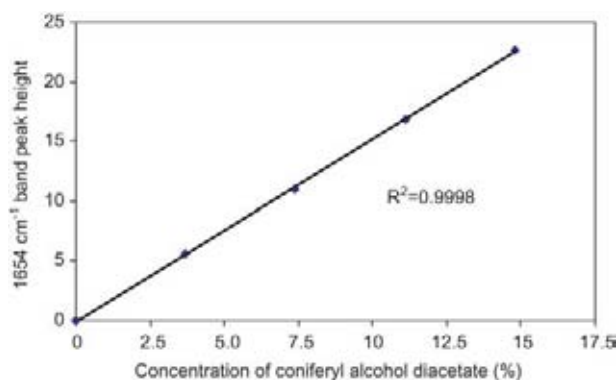
### Comparing Raman and NMR measurements based on models

The lignin models, coniferyl alcohol diacetate (**VI**) and a G-DHP (**III**), were investigated. In the diacetate NMR study,  $\text{CDCl}_3$  served as solvent. The G-DHP was analyzed in dioxane as solvent. Both these solvents are suitable for Raman studies, because in the spectral region of interest no interfering solvent bands are present.

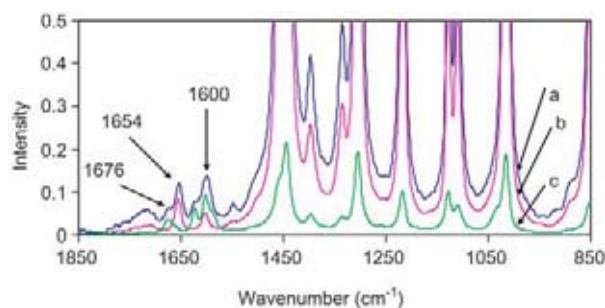
**Coniferyl alcohol diacetate (VI)** Varying concentrations of **VI** were prepared in  $\text{CDCl}_3$  and analyzed by Raman and  $^{13}\text{C}$  NMR. Raman measurements at  $1654\text{ cm}^{-1}$  correlated very well with the concentration data (Figure 5). In Raman spectroscopy, peak height was measured after normalizing the spectra on the band of  $\text{CDCl}_3$  at  $2252\text{ cm}^{-1}$ .

**Quantification of coniferyl alcohol units in G-DHP (III)** Although DHPs prepared from coniferyl alcohol are structurally somewhat different than native lignins or MWLs, they are helpful in structural studies of lignins. In the context of this work, a particular G-DHP (**III**) was chosen because it had a high concentration of coniferyl alcohol end groups. A detailed structural  $^{13}\text{C}$  NMR comparison of **III** with loblolly pine MWL has been published (Terashima et al. 1995). The aim of the present study was to check the applicability of the Raman analysis, based on the  $\text{C}=\text{C}$  stretch mode band, in direct comparison to the same sample analyzed by  $^{13}\text{C}$  NMR. Raman spectra in dioxane were recorded from the compounds: **III** (0.0654 M), Figure 6a; **II** (0.023 M), Figure 6b; and **I** (diluted concentration), Figure 6c.

The  $\text{C}=\text{C}$  stretch band in **III** ( $1654\text{ cm}^{-1}$ ) has a shoulder at  $1676\text{ cm}^{-1}$  because of the coniferaldehyde  $\text{C}=\text{O}$  group. However, considering that the weak shoulder is in distance of  $22\text{ cm}^{-1}$  from the  $1654\text{ cm}^{-1}$  maximum, its influence on the coniferyl alcohol determination based on  $1654\text{ cm}^{-1}$  band is probably minimal (Figure 6a). The possible influence of the shoulder at  $1654\text{ cm}^{-1}$  was also assessed by deconvolution. It was found that the contribution of the shoulder is around 1.25% to the  $1654\text{ cm}^{-1}$  band intensity. Raman spectra of **III** and **II** (Figure 6a and b) showed, as expected, the  $\text{C}=\text{C}$  band at  $1654\text{ cm}^{-1}$ . Based on the  $1654\text{ cm}^{-1}$  band intensity, the con-



**Figure 5** Calibration line for quantitation of coniferyl alcohol diacetate (**VI**) in  $\text{CDCl}_3$  by Raman ( $1654\text{ cm}^{-1}$  band) and  $^{13}\text{C}$  NMR (x-axis).



**Figure 6** Raman spectra of G-DHP (**III**) (a), coniferyl alcohol (**II**) (b), and coniferaldehyde (**I**) (c) in dioxane. Spectra are shifted on the intensity scale for display purposes. The  $1654\text{ cm}^{-1}$  band of the DHP was used for quantification. All peaks below  $1475\text{ cm}^{-1}$  in the spectra are due to dioxane.

centration of the coniferyl alcohol units in **III** was found to be 28.8% (see the calculation below). This value is higher compared to the quantitative  $^{13}\text{C}$  NMR results ( $25.8 \pm 0.5\%$ ). Despite the small difference of 3%, the agreement between the Raman and NMR determinations is quite good.

### Raman calculation for quantifying coniferyl alcohol residues in G-DHP (III)

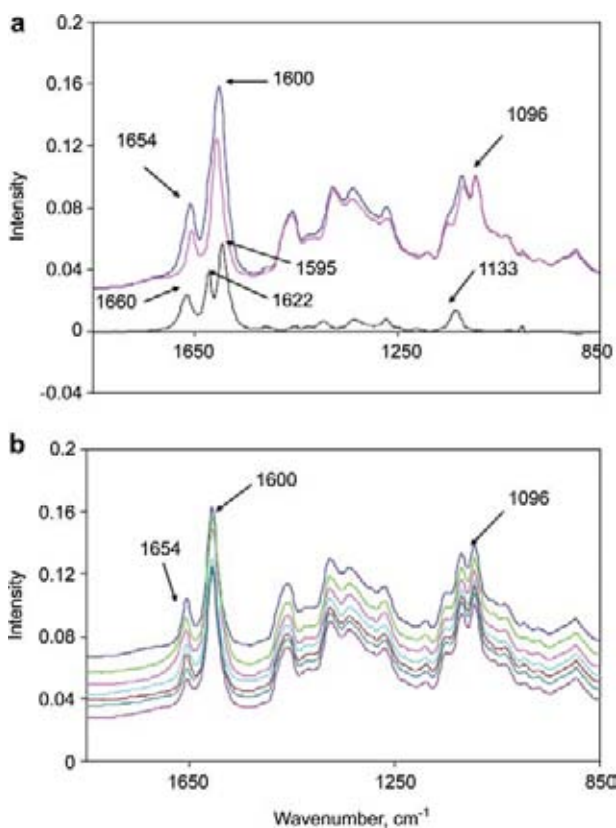
$$\text{Molarity fraction (MF)} = (0.818 \times 0.023) / 0.0654 \quad (1)$$

$$\% \text{ Coniferyl alcohol} = \text{MF} \times 100 \quad (2)$$

where 0.818 in Eq. (1) is the multiplication factor needed to make the relative intensity of coniferyl alcohol's  $1654\text{ cm}^{-1}$  band ( $I_{1654}/I_{1015}$ ) equal to that of G-DHP's ( $I_{1654}/I_{1015}$ ), 0.023 in Eq. (1) is the molarity of coniferyl alcohol, 0.0654 in Eq. (1) is the molarity of **III** (based on  $\text{C}_9$  lignin unit weight of 185), the MF that produces a ( $I_{1654}/I_{1015}$ ) band intensity equivalent to  $0.818 \times$  coniferyl alcohol ( $I_{1654}/I_{1015}$ ).

### Spruce wood

**Coniferaldehyde residues** In lignin, only coniferaldehyde has a band at  $1133\text{ cm}^{-1}$ , and therefore removal of other constituents is not required before the Raman determination of coniferaldehyde groups. In the lignocellulosic matrix, however, bands due to cellulose influence this region. Therefore, only indirect measurements are possible. One possibility is to remove coniferaldehyde structures by means of lignin-retaining bleaching and recording a difference Raman spectrum "intensity of the band at  $1133\text{ cm}^{-1}$  before bleaching minus intensity of the band at  $1133\text{ cm}^{-1}$  after bleaching" (Figure 7a). For various bleaching experiments several differential spectra were recorded. Figure 7a demonstrates the method by taking the borohydride-bleached case, as an example. The intensity of the  $1133\text{ cm}^{-1}$  band height was measured and ratioed against the  $1096\text{ cm}^{-1}$  holocellulose band ( $I_{1133}/I_{1096}$ , Table 2, column 3). Data resulting from difference spectra are provided in Table 2 for  $\text{H}_2\text{O}_2$  bleached and  $\text{NaBH}_4$  bleached woods. Similar values were obtained in other bleaching experiments (data not shown). Each of the presented two values was divided



**Figure 7** (a) Effect of  $\text{NaBH}_4$  bleaching on the Raman spectrum of spruce wood: top, unbleached; middle, bleached, and bottom, difference. Contribution removed at  $1133\text{ cm}^{-1}$  is due to coniferaldehyde structures in wood. Additionally, although contributions removed at  $1595$ ,  $1622$ , and  $1660\text{ cm}^{-1}$  are due largely to the same residues, contributions of other chromophores (quinones) are present as well. (b) Spectra of differently bleached spruce-wood samples. From top to bottom, the spectra represent samples that were bleached by different sequences. When spectra are normalized on the  $1096\text{ cm}^{-1}$  band, significant differences are seen in the  $1654\text{ cm}^{-1}$  band intensity (Table 2, column 5, sample nos. 5–11).

by 1.46 to correct for the difference in the holocellulose concentration of wood (68%) and BSKP (99%). Such “corrected ( $I_{1133}/I_{1096}$ )” data are presented in Table 2 (column 4). Last, to calculate the coniferaldehyde concentration in wood, the “corrected ( $I_{1133}/I_{1096}$ )” was divided by the ( $I_{1133}/I_{1096}$ ) data for the 1% coniferaldehyde in BSKP sample (Table 2, sample no. 1, column 3). A value of 1.1% (Table 2, sample no. 3, column 7) was obtained from difference spectra (unbleached wood minus  $\text{H}_2\text{O}_2$  bleached wood). A similar calculation of the type (unbleached wood minus  $\text{NaBH}_4$  bleached wood) resulted in a value of 1.0% (Table 2, sample no. 4, column 7).

Accordingly, alkaline peroxide and the borohydride treatments of wood were equally effective in destroying/reducing the coniferaldehyde structures. In fact, the borohydride treatment is expected to lead to elevated amounts of coniferyl alcohol groups (Johnson and Rickborn 1970). In that case, an enhancement of the  $1654\text{ cm}^{-1}$  Raman band intensity should be expected (Table 2, sample no. 6, column 8 data), where coniferyl alcohol concentration in bleached spruce samples is reported and supports this argument: in the borohydride-bleached sample, the concentration of coniferyl alcohol was approximately twice of that in sample numbers 7 and 11. In these extensively bleached pulps,  $\text{NaBH}_4$  was not the first bleaching stage.

Based on the results listed in Table 2, the amount of coniferaldehyde groups in spruce wood is 1.05% (average of the two measurements). This value recalculated for the lignin (Klason residue in spruce 28%, Pettersen 1984) results in 3.8% coniferaldehyde content in lignin. This Raman-determined value matches excellently with the reported data of 4% based on UV and  $^{13}\text{C}$  NMR spectroscopy analyses of spruce MWL (Aulin-Erdtman and Hegbom 1958; Marton and Adler 1961; Dence 1992; Capanema et al. 2004).

**Coniferyl alcohol** The band of coniferyl alcohol at  $1654\text{ cm}^{-1}$  is influenced by coniferaldehyde and quinones

**Table 2** Lignin model band intensities in BSKP and ethylenic residue concentration in wood.

1	2	3	4	5	6	7	8
Sample no.	Substance, spectrum type, and bleaching step	$I_{1133}/I_{1096} \pm \text{SD}$	$I_{1133}/I_{1096}$ Corrected <sup>a</sup>	$I_{1654}/I_{1096} \pm \text{SD}$	$I_{1654}/I_{1096}$ Corrected <sup>a</sup>	Coniferaldehyde (%)	Coniferyl alcohol (%)
1	1% coniferaldehyde in BSKP <sup>b</sup>	$0.166 \pm 0.018$	–	–	–	1.0	–
2	1% coniferyl alcohol in BSKP	–	–	$0.131 \pm 0.009$	–	–	1.0
3	(Unbleached minus $\text{H}_2\text{O}_2$ bleached <sup>c</sup> spruce	0.266	0.182	–	–	1.1	–
4	(Unbleached minus $\text{NaBH}_4$ -bleached) spruce	0.244	0.167	–	–	1.0	–
5	$\text{H}_2\text{O}_2$ black spruce	–	–	0.305	0.208	–	1.59
6	$\text{NaBH}_4$ black spruce	–	–	0.397	0.272	–	2.08
7	5 + $\text{NaBH}_4$ spruce	–	–	0.188	0.129	–	0.98
8	6 + $\text{H}_2\text{O}_2$ black spruce	–	–	0.315	0.216	–	1.65
9	5 + $\text{H}_2\text{O}_2$ black spruce	–	–	0.319	0.218	–	1.66
10	8 + $\text{NaBH}_4$ spruce	–	–	0.314	0.215	–	1.64
11	7 + $\text{H}_2\text{O}_2$ black spruce	–	–	0.183	0.125	–	0.95

<sup>a</sup>Corrected by dividing the ( $I_{1133}/I_{1096}$ ) band intensities by 1.46 so they can be directly compared with the BSKP data.

<sup>b</sup>BSKP, bleached softwood kraft pulp.

<sup>c</sup> $\text{H}_2\text{O}_2$ -bleached is alkaline  $\text{H}_2\text{O}_2$ -bleached.

and other chromophores. The challenge is the removal of these influences before calculating the concentration of coniferyl alcohol in wood. Lignin retaining bleaching is a good possibility. It is known that such bleaching does not remove coniferyl alcohol units (Agarwal and Atalla 1993; Agarwal and McSweeney 1997). The bleaching sequences are listed in Table 2 for sample numbers 5 to 11. The Raman spectra of the bleached samples are presented in Figure 7b.

Upon bleaching, the fluorescence signal underlying the Raman spectra declined significantly, indicating that long-wavelength-absorbing chromophores were removed. Compared to the reductive bleaching, such decline was more prominent in the alkaline  $H_2O_2$  bleached samples. The treatment that produced the lowest  $1654\text{ cm}^{-1}$  band intensity in the spectrum was most successful in removing contributions of non-coniferyl alcohol entities and therefore can be taken as representing Raman intensity of the coniferyl alcohol units. As Table 2 shows, the corresponding multi-stage bleaching sequence is alk.  $H_2O_2 \rightarrow NaBH_4 \rightarrow$  alk.  $H_2O_2$ . Once again, as was the case for the coniferaldehyde calculation, the  $1654\text{ cm}^{-1}$  peak intensity was ratioed to the cellulose band at  $1096\text{ cm}^{-1}$  and corrected for the holocellulose difference. These values are listed in Table 2 in columns 5 and 6. Although all percentage coniferyl alcohol values are given in each case in column 8, the lowest values (in our case 0.95%) are probably the most accurate ones. The conversion of this minimum value for lignin results in 3.4% coniferyl alcohol. Once again, this number is in good agreement with the corresponding UV data of 3% for spruce MWL (Aulin-Erdtman and Heggbon 1958; Marton and Adler 1961; Dence 1992).

The coniferyl alcohol concentration of 0.95% in wood obtains further support from the result of sample number 6, where an apparent coniferyl alcohol concentration (column 8) is found to be 2.08%. It is approximately twice the actual concentration, because upon borohydride treatment 1% of the wood-coniferaldehyde units were effectively converted to coniferyl alcohol units and thereby contributed to the intensity of the  $1654\text{ cm}^{-1}$  band. The consequences for Raman determination of coniferyl alcohol are that in the presence of coniferaldehyde units  $NaBH_4$  bleaching should not be the first bleaching stage in a multi-stage sequence. That is because  $NaBH_4$  converts the existing coniferaldehyde units to coniferyl alcohol units and artificially enhances the latter's concentration.

Another source of error could be that bleaching under alkaline  $H_2O_2$  conditions usually give rise to formation of other chromophores (hydroxylated *p*-quinone is one possibility) (Rundlof et al. 2006) and, moreover, organic acids, vanillin, and hydroquinones can arise (Gellerstedt et al. 1980). Some of these newly formed structures can account for the observed  $1654\text{ cm}^{-1}$  band-intensity decline (38%, Table 2, column 5) when the peroxide-treated sample 5 was further bleached by  $NaBH_4$  (sample 7). The probability is high that upon peroxide bleaching some of the newly generated chromophores contributed to the intensity of  $1654\text{ cm}^{-1}$  band. As a consequence, the intensity of the  $1654\text{ cm}^{-1}$  band did not decline to the extent as should have been upon coniferaldehyde de-

struction alone. Nevertheless, these new chromophores were such that they could be reduced or modified by the borohydride (ketones, aldehydes, or quinones), as suggested by the data for sample 7 (Table 2, column 5).

### Spruce TMP

Previously, an FT-Raman method has been applied to study the bleaching of spruce TMP (Agarwal and Landucci 2004). The TMP was brightened to varying degrees depending upon the bleaching sequences used. The TMPs were analyzed according to Agarwal and Landucci (2004) and the results are listed in Table 3. As was the case for the wood samples, data in Table 3 (column 5) indicated variable coniferyl alcohol concentration depending upon the extent to which other  $1654\text{ cm}^{-1}$  contributing structures existed in the pulp.

Additionally, Table 3 contains the results obtained from the difference spectra "unbleached TMP minus alkaline peroxide bleached TMP", the calculated concentration of coniferaldehyde (TMP no. 2, column 4). The data are shown only for the TMP no. 2, because upon further bleaching no additional coniferaldehyde structures were removed (see Figure 6 in Agarwal and Landucci 2004). However, that was not the case for the coniferyl alcohol concentrations, which declined upon additional brightening; an observation similar to the case of bleached-wood samples (Table 2, column 8). Once again, the reason for the concentration decline was not the removal of the alcohol residues but rather removal of the contributions at  $1654\text{ cm}^{-1}$  that did not originate in coniferyl alcohol units. The lowest value of 0.72% in Table 3 (column 5, TMP no. 9) is expected to be the best representative concentration of the coniferyl alcohol units in TMP.

Data in Table 4 are suited for comparison of ethylene groups in wood and TMP. It is obvious that TMP contains less coniferaldehyde and coniferyl alcohol than wood. The concentrations of these groups declined by 28% and 24%, respectively. The decline seems to be associated with the thermally induced structural changes in spruce-wood lignin and this interpretation is supported by the

**Table 3** Concentrations of coniferaldehyde and coniferyl alcohol residues in TMP.

1	2	3	4	5
TMP #	Description	Brightness (%)	Coniferaldehyde <sup>a</sup> (%)	Coniferyl alcohol <sup>b</sup> (%)
1	Unbleached	52.2	–	2.88 <sup>c</sup>
2	(Unbleached minus $H_2O_2$ bl)	–	0.76	–
3	$H_2O_2$	71.8	–	1.45 <sup>c</sup>
4	$3 + H_2O_2$	77.5	–	1.17 <sup>c</sup>
5	$3 + Na_2S_2O_4$	70.4	–	1.33 <sup>c</sup>
6	$4 + NaBH_4$	75.2	–	0.89 <sup>c</sup>
7	$5 + H_2O_2$	78.8	–	0.75 <sup>c</sup>
8	$6 + Na_2S_2O_4$	79.0	–	0.80 <sup>c</sup>
9	$7 + NaBH_4$	79.9	–	0.72

<sup>a</sup>Based on the band ratio  $I_{1133}/I_{1096}$ .

<sup>b</sup>Based on the ratio  $I_{1654}/I_{1096}$ ; values higher than 0.72 are due to contributions from non-coniferyl alcohol entities.

<sup>c</sup>Coniferyl alcohol values for 1 and 3–8 are artificially high due to  $1654\text{ cm}^{-1}$  contributions from other entities.

**Table 4** Comparison of ethylenic residue concentrations.

Sample	Coniferaldehyde		Coniferyl alcohol	
	(%)	b.o. lignin (%)	(%)	b.o. lignin (%)
Spruce wood	1.05	3.75	0.95	3.40
Spruce TMP	0.76	2.71	0.72	2.57
Percentage change (wood-TMP)	28	28	24	24

FT-Raman investigations of heat-treated woods (Yamauchi et al. 2005) and hydrothermally degraded ground wood paper (Proniewicz et al. 2002). These researchers found that the ethylenic structures undergo significant changes upon heating.

## Conclusions

An FT-Raman method based on spectra recorded before and after bleaching was successfully used to quantify the ring-conjugated ethylenic structures in spruce wood and TMP lignins. In the case of wood lignin, the Raman results were in excellent agreement with the known values for ethylenic structures in spruce MWL. For TMP, such data are not available in the literature. Compared to spruce wood, the concentrations of coniferaldehyde and coniferyl alcohol groups in TMP are 28% and 24% lower, respectively. FT-Raman spectroscopy is well suited for in situ quantification of such groups in woods and mechanical pulps.

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