

Estimation of S/G ratio in woods using 1064 nm FT-Raman spectroscopy

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ABSTRACT

Two simple methods based on the 370 cm⁻¹ Raman band intensity were developed for estimation of syringyl-to-guaiacyl (S/G) ratio in woods. The methods, in principle, are representative of the whole cell wall lignin and not just the portion of lignin that gets cleaved to release monomers, for example, during certain S/G chemical analyses. As such, it is like the whole-cell-wall NMR methods. The Raman analysis is quick, free of the use of harmful chemicals, carried out nondestructively, and is insensitive to the wet or dry state of the sample. The only limitation is that a wood sample should not be significantly fluorescent, although this can be rectified in some cases. The reliability of the Raman approach was first tested by the quantitative analysis of several syringyl lignin models by sampling them, separately, in dioxane and in avicel. Good linear correlations between 370 cm⁻¹ band intensity and model concentrations were obtained. Subsequently, based on 370 cm⁻¹ intensity, S contents of woods were determined. To test the accuracy of the Raman methods, the obtained syringyl contents in woods were calibrated against the S/G values generated by thioacidolysis, DFRC, and 2D-HSQC NMR methods. The former two are S/G methods that take into account only the monomers cleaved from β -O-4-linked lignin units whereas NMR reports S/G ratio on the whole cell wall lignin.

KEYWORDS

Cell wall, DFRC, Guaiacyl lignin, NMR, Raman, S/G ratio, Syringyl lignin, Thioacidolysis

INTRODUCTION

Understanding of wood lignins is important for a number of reasons. Lignin plays a significant role in the growth and development of plants and is also important to the industrial utilization of various types of biomass. Lignin is biosynthesized from the polymerization of the three types of *p*-hydroxycinnamyl alcohols that produce *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units in the polymer. In industries, from pulp and paper to bioethanol, lignins play an important role.[1,2] Successful manipulation of its structures can have beneficial outcomes for several biomass-based technologies. For instance, technologies focused on improving the sugar release for biofuel production,[3] improved delignification in pulping and

bleaching,[4] digestibility of forages in ruminants,[5] and valorization of lignin into high-value products.[6]

The S/G ratio is an important parameter for biomass characterization. Considering that lignin composition can influence the utilization of biomass, various techniques have been used to estimate the H, G, and S monomer composition of lignins. Such techniques consist of both wet chemical techniques (e.g., thioacidolysis and nitrobenzene oxidation), and spectroscopic methods (e.g., 2D-HSQC NMR, FT-IR, and NIR). Moreover, whereas 2D-HSQC NMR analyzes the whole cell wall lignin, most other methods only analyze the monomers generated by releasing monomers by cleaving β -O-4-bonds in lignin units.[7] Therefore, the latter approaches are limited in that the monomer composition information provided is not necessarily representative of whole cell wall lignin. Moreover, the chemical methods can be time consuming and labor intensive and require use of expensive and unsafe chemicals.

1064 nm FT-Raman is a technique in the field of Raman spectroscopy that has unique advantages.[8] In the context of lignocellulose materials, spectra with minimum fluorescence and high signal-to-noise ratio are obtained.[9] 1064-nm-excited Raman spectra of wood milled-wood lignins (MWLs) and lignin models have been reported previously.[10,11] In hardwood MWLs and S lignin models, a band at 370cm⁻¹ was identified that was significantly more intense compared to that of softwood MWLs and models of G and H lignins.[10,11] Raman spectra of hardwood and softwood MWLs are compared in Fig. 1. In the present investigation which focused on the estimation of S/G ratio in woods, one softwood and seven hardwoods were used (Table 1). The S content in woods was estimated using Raman and was based upon the intensity of 370cm⁻¹ band. This Raman data was then correlated with the values of S/G ratios obtained using thioacidolysis, DFRC (derivatization followed by reductive cleavage), and 2D-HSQC NMR. Additionally, to further verify the Raman approach, a number of S and G lignin models were quantified by Raman spectroscopy in dioxane and avicel matrices.

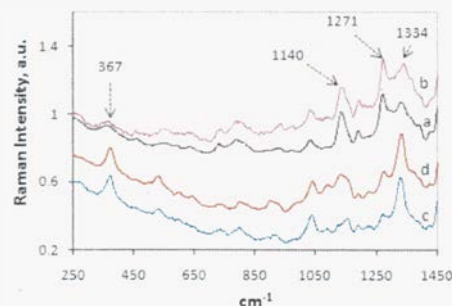


Figure 1. 1064-nm-excited Raman spectra of MWLs in the region 250 - 1450 cm⁻¹; (a) spruce, (b) loblolly pine, (c) aspen, (d) sweet gum.

The Raman methods are expected to provide S content based on whole cell wall lignin. The technique is simple, direct, and quick. Wet or dry sample-state

does not matter. Only a small amount of sample is needed, and it can be recovered after the analysis.

To develop the S/G ratio method based on Raman, the woods listed in Table 1 were selected. Their lignin contents were determined using the acetyl bromide method.[12]

Table 1: Characteristics of the woods used

Sample	Type	% lignin ^a
Black spruce	Softwood	30.0
Aspen	Hardwood	15.1
Cottonwood	Hardwood	25.7
Hickory	Hardwood	23.2
Madrone	Hardwood	17.7
Sweetgum	Hardwood	19.8
White Birch	Hardwood	15.4
Willow	Hardwood	24.4

^aBased on acetyl bromide method [12]

RESULTS AND DISCUSSION

Estimation of S groups in Lignin models. First, using the 370 cm⁻¹ band intensity method, 3 different S-group-containing models were quantified in the concentration range of 1.25 to 20% (w/w) in dioxane and avicel. Good linear correlations between 370 cm⁻¹ band intensity and the model concentrations were obtained in all three cases (Figs. 2 and 3).

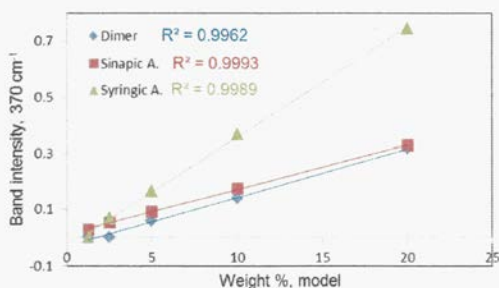


Figure 2. Quantitation of S lignin models in dioxane using Raman spectroscopy. The models were syringylglycol- β -guaiacyl ether (dimer), sinapic acid, and syringic acid.

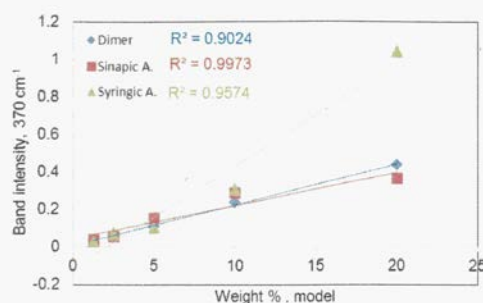


Figure 3. Quantitation of S lignin models in avicel using Raman spectroscopy. The models were syringylglycol- β -guaiacyl ether (dimer), sinapic acid, and syringic acid.

However, to mimic the wood cellulose matrix, when syringic acid and the “dimer” were analyzed in avicel, from the intensity variation at 370 cm⁻¹ it was noted that the two models were somewhat heterogeneously distributed. This may have to do with these models’ particular structural characteristics. Therefore, for these, the average intensity in Fig. 3 was calculated from 10 (instead of 3) different locations on the sample pellet. The heterogeneous sample distribution was the reason why the correlation coefficients in avicel were lower (Fig. 3) compared to the corresponding constants in dioxane (Fig. 2).

Estimation of S groups in woods. Raman spectra with good S/N ratio were obtained from different wood pellets. The pellets were produced from each of the woods that were first Wiley milled and extracted. A second set of pellets was made from the same woods but they were first mostly delignified. This was done to carry out intensity calculation at 370 cm⁻¹ by first normalizing the spectra at 1096 cm⁻¹ (cellulose band) and subsequently, subtracting the spectrum of the delignified wood from that of the non-delignified wood {Method 1: (control - delig.)}. Another way the band intensity at 370 cm⁻¹ was calculated was by subtracting the spectrum of black spruce (BS) from the spectrum of each of the hardwoods used in the study {Method 2: (wood - BS)}. Method 2 works because there is very weak intensity present at 370 cm⁻¹ in the spectrum of BS (Fig. 1) - independent of Raman, it is known that BS, a softwood, is devoid of any S lignin.[13] The use of the two approaches to calculate 370 cm⁻¹ band intensity (Method 1 and Method 2) was further justified by the strong correlation ($R^2 = 0.96$) that was found between the band intensities that were estimated from the two Methods (Fig. 4). Lastly, the 370 cm⁻¹ Raman peak intensities were corrected for differences in lignin amount between the hardwoods.

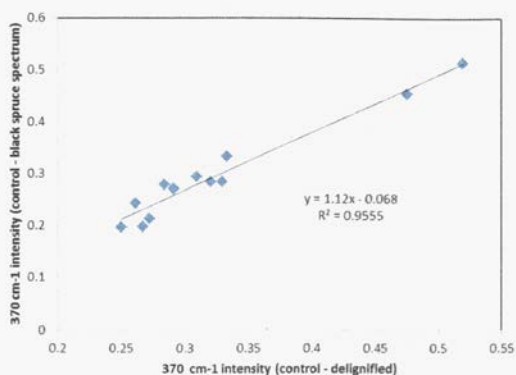


Figure 4. Correlation of the 370 cm⁻¹ band intensities estimated using Method 1 (control - delignified) and Method 2 (control - black spruce). Several hardwoods including those used here were included.

Estimation of S groups by thioacidolysis, DFRC, and 2D-HSQC NMR. The 7 hardwoods used in the Raman investigation were also subjected to S and S/G ratio analyses by the often-used methods of thioacidolysis,[7] DFRC,[14] and 2D-HSQC NMR.[15] For the woods, good correlations were obtained ($R^2 = 0.92$ and 0.99 for 2D-HSQC NMR vs. DFRC and thioacidolysis vs. 2D-HSQC NMR) between the data that was generated using the three methods (Fig. 5).

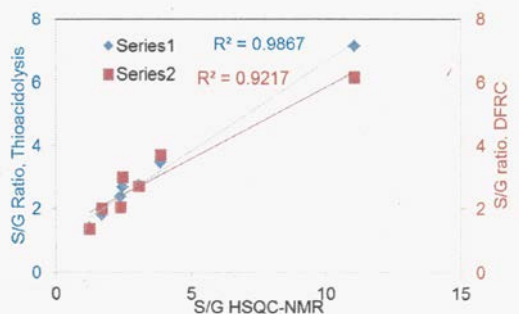


Figure 5. Linear correlations between methods for estimating S/G ratio of hardwoods; thioacidolysis vs. 2D-HSQC NMR and DFRC vs. 2D-HSQC NMR.

When S content data from Raman were correlated against the S/G values from each of the above-mentioned methods the plots shown in Figs. 6 - 8 were obtained.

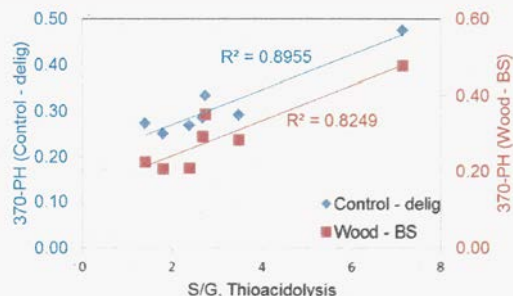


Figure 6. Correlations between Raman S contents and thioacidolysis S/G ratios of hardwoods.

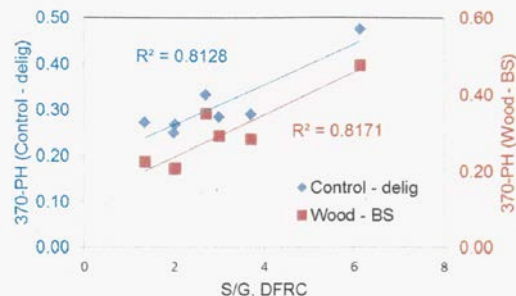


Figure 7. Linear correlations between Raman S contents and DFRC S/G ratios of hardwoods.

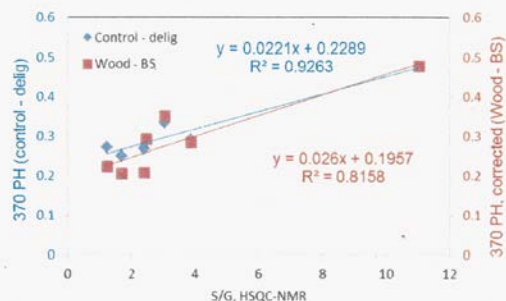


Figure 8. Linear correlations between Raman S contents and 2D-HSQC NMR S/G ratios of hardwoods.

The correlations between the methods are summarized in Table 2. From the Table it is evident that the Raman method correlated well with the other often-used methods (R^2 between 0.81 and 0.93) with the highest correlation (0.93) seen between the 2D-HSQC NMR and Raman Method 1 (Table 2). That is to be expected because both the NMR and Raman methods analyzed syringyl content of whole cell walls.

Table 2: Correlation coefficients based on comparison of the two Raman methods with traditional methods

Method	Raman	2D-HSQC NMR	Thioaci.	DFRC
Raman, Method 1 ^a	—	0.926	0.896	0.813
Raman, Method 2	—	0.816	0.825	0.818
HSQC-NMR		—	0.987	0.922
Thioacid.			—	ND ^b
DFRC				—

^aMethod 1 and Method 2 are based on how the Raman

spectra were processed for calculating the 370 cm⁻¹ band

intensity. Method 1, (Hardwood - delignified hardwood);

Method 2, (Hardwood black spruce).

^bND is not done

CONCLUSIONS

To estimate S/G ratios, two Raman methods were developed that were both based on the intensity of the 370 cm⁻¹ band in the Raman spectra of hardwoods. Both methods were found to correlate well with the established methods that are generally used to calculate the S/G ratio in woods. However, in addition to being convenient, the main advantage of the Raman methods was, like 2D-HSQC NMR, the ability to provide the estimation based on the whole cell wall. Therefore, the Raman methods developed in this work appear to be useful for estimating S/G ratios in woods.

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