CELLULOSE CRYSTALLINITY OF WOODS, WOOD PULPS, AND AGRICULTURAL FIBERS BY FT-RAMAN SPECTROSCOPY

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ABSTRACT

Recently, for estimating cellulose crystallinity, the authors had proposed univariate and multivariate FT-Raman methods. In the present investigation, the univariate method was applied to a number of additional biomaterials (softwoods, hardwoods, wood pulps, and agricultural fibers). The materials were studied in the native, partially-delignified, and alkaline hydrogen peroxide bleached states. The latter two states were examined to evaluate how Raman crystallinity measurement is influenced by the presence of lignin in the sample and by the existence of sample-fluorescence in the Raman spectrum. It was found that for most lignin containing materials the univariate Raman method was a suitable alternative to calculate cellulose crystallinity. Additionally, another univariate FT-Raman method based on Segal-WAXS (or 18-WAXS) was developed (18-univariate Raman) and compared to the one proposed previously (21-univariate Raman). Compared to 21-univariate Raman, the 18-univariate Raman method produced the crystallinities that were about 17% higher. Therefore, it is proposed that for estimating cellulose crystallinities in lignocellulosic materials 21-univariate Raman be used instead of 18- or Segal-WAXS method.

Keywords: cellulose crystallinity, FT-Raman, WAXS, wood, lignocellulosics, lignin

INTRODUCTION

To reliably determine cellulose crystallinity, the authors have proposed two new FT-Raman methods[1]. It was shown that both the univariate and multivariate methods produced good correlations with crystallinities of the calibration set cellulose samples and that the Raman methods were better compared to the modified-Segal-WAXS method (called Segal WAXS in the publication[1]). However, the modified-Segal-WAXS method differed from the traditional Segal-WAXS[2] by the fact that the contribution of the amorphous cellulose was measured at $2\theta = 21^{\circ}$ and subtracted from the [002] peak intensity[1]. The reasoning for this was that subtraction at 21° was more appropriate because that's where the peak position of the amorphous cellulose really existed. As expected, this approach resulted in lower values of cellulose crystallinity compared to the Segal-WAXS. Nevertheless the Raman crystallinity values were reliable in the entire crystallinity range. Moreover, when these methods were applied to measure crystallinities of milled Whatman CC31 samples, Whatman CC41, Whatman 541, CF-11, and Avicel PH-101 reasonable values were obtained[1].

It is well known that in materials where lignin, hemicellulose, and pectins are present, their contributions cause extra X-ray scattering and lead to an inaccurate measurement of the cellulose crystallinity[3]. These amorphous materials affect both the width of the [002] peak of the crystalline cellulose and the background under the cellulose diffraction region. There is no simple way to account for such interference/overlap or remove the contributions of the non-cellulosic components. Similar problems were also encountered in other methods (for a brief review see[1]).

Therefore, the objective of the present study was to use the 21-univariate Raman method to determine cellulose crystallinity of lignin containing materials. A large number of materials, fairly heterogeneous in composition, were selected. The materials consisted of softwoods, hardwoods, wood pulps, and agricultural fibers.

EXPERIMENTAL

Materials

Materials investigated in the present project are listed in Table 1. The crystallinities of Wiley-milled (40 mesh) materials were measured in 3 states – after extraction with acetone: H_2O (9:1), after partial delignification with acid chlorite, and after alkaline peroxide bleaching (lignin retaining bleaching). The standard procedures were used in such treatments.

FT–Raman

All samples were analyzed with a Bruker MultiRam spectrometer (Bruker InstrumentsInc., Billerica, Massachusetts). This Raman system is equipped with a 1,064nm 1,000mW continuous wave (CW) diode pumped Nd:YAG laser. Approximately 0.20 g of each sample was pressed into a pellet with the help of a hydraulic press. The laser power used for sample excitation was 600 mW, and 1,024 scans were accumulated. Bruker's OPUS software program was used to find peak positions and process the spectral data. The processing of the spectra was identical to what has been previously described[1]. For the identical samples, the 380/1096 band intensity ratios obtained in MultiRam were lower compared to those in RFS-100 (the FT-Raman instrument used in[1]). Therefore, to compensate for this

instrument dependence, the RFS-100 equivalent values for the former were obtained by using a multiplication factor.

X-ray

Wide-angle X-ray diffraction profiles were recorded on a Bruker X-ray diffractometer with a Hi-Star 2-D area detector at the Materials Research Science and Engineering Center, University of Wisconsin, Madison. Diffractograms were obtained on the same sample pellets that were analyzed in FT–Raman. Crystallinity was calculated as described in the following section.

Crystallinities

For each of the 41 samples WAXS crystallinities were calculated in two ways - by subtracting the amorphous contribution at $2\theta = 21^{\circ}$ and 18° (called 21-WAXS and 18-WAXS, respectively; Table 1). Therefore, the 18-WAXS method was same as the Segal-method[2].

Two different univariate-Raman methods were used for calculating the Raman crystallinities. The two Raman methods were dependent upon the two different reference WAXS crystallinity values (21-WAXS and 18-WAXS) of the control Whatman CC31 sample. Both methods were based on the independent calibrations between the 380/1096 Raman band intensity ratios (from the FT-Raman spectra of the calibration set samples) against the calculated calibration set crystallinities of the cellulose samples. The first method was same as described earlier by the authors[1] and was based, in part, on the 21-WAXS crystallinity of the control Whatman CC31 sample. The second method was developed during this study (see latter) and differed (from the first method) essentially in that instead of the 21-WAXS the 18-WAXS crystallinity data was used for the control Whatman CC31 sample. All other steps between the two calibrations were similar.

Table 1. WAXS crystallinities

Sample #	Sample ID	21-WAXS /%	18- or Segal-WAXS /%	% Lower Compared to Segal
Softwoods				
1	Black spruce	19.3	56.5	65.8
2	Engelmann spruce	20.9	56.9	63.3
3	Jack pine	16.3	55.5	70.6
4	Loblolly pine	16.5	52.2	68.4
5	Lodgepole pine	17	62.6	72.8
6	Red cedar	16.5	52.2	68.4
7	Red pine	17	58.3	70.8
8	Sitka spruce	20.3	56.5	64.1
9	Western Hemlock	18.9	55.5	66.0
Hardwoods				
10	American elm	17.2	58.6	70.7
11	Aspen	25.8	60.8	57.6
12	Cottonwood	19.3	61.4	68.6
13	Dogwood	12.6	59.9	79.0
14	Hickory	16.1	60.6	73.4
15	Madrone	12.2	62.2	80.4
16	Red maple	17.5	58.1	69.9
17	Sour orange	9.2	65.4	85.9
18	Sweetgum	13.2	58.8	77.6
19	White birch	14.3	55.1	74.0
20	Willow	15.8	61.7	74.4
21	Eucalyptus	14.2	56.6	74.9
Ag residues				
22	Alfalfa	14.5	56.7	74.4
23	Corn stalk	14	59.3	76.4
24	Kenaf	22.5	65.7	65.8
25	Kenaf core	13.8	56.3	75.5
26	Oat straw	17.2	66.1	74.0
27	Sugarcane bagasse	6.3	51.1	87.7
28	Flax	48.2	78	38.2
29	Ramie	62.8	86.6	27.5
30	Banana stem	2.3	44.5	94.8
Pulps				
31	Unbleached softwood kraft pulp	44.7	76.5	41.6
32	Bleached softwood kraft pulp	40.4	70	42.3
33	Bleached hardwood kraft pulp	38.5	70.4	45.3
34	Spruce TMP	29.5	64.2	54.1
35	Hemlock CTMP	35.8	62.6	42.8
Compression vs. opposite woods				
36	Red pine compression wood	19.5	60.7	67.9
37	Red pine opposite wood	24.3	58.4	58.4
Heartwood vs. sapwood				
38	White oak heartwood	12.3	54.6	77.5
39	White oak sapwood	11.8	54	78.2
40	Pacific yew heartwood	17	55.7	69.5
41	Pacific yew sapwood	21.4	54.7	60.9

RESULTS AND DISCUSSION

WAXS crystallinities

From Table 1, it is clear that compared to 18-WAXS 21-WAXS crystallinities were significantly lower (column 3, anywhere from 95% to 28%). Although somewhat lower values were expected for the 21-WAXS due to the fact that a higher amorphous intensity contribution is removed in the calculation, the reduction is quite severe particularly for the samples containing higher amount of lignin and hemicelluloses. This has to do primarily with the broadness of the [002] peak in the diffractograms of these materials because of which the contribution at 21° remains fairly high. This interpretation is supported by the X-ray diffractograms of avicel and loblolly pine (# 4) shown in Fig. 1. Moreover, when Table 1 data is compared between different categories of materials, no category dependence behavior is noted and the difference seems to be dependent upon the composition of the materials. Agriculture residues showed the most % decline variation (Table 1, column 3) from 28% for ramie (# **29**) to 88% for sugarcane bagasse (# **27**). Materials that have high cellulose content and/or had low amounts of hemicellulose and lignin showed the least difference (ramie – 28%, flax – 38%, and wood pulps #s **31** to **33**, and **35%** ~ 42% to 45%). This is not surprising given that as amorphous materials lignin and hemicelluloses are known to contribute broadly in the regions of cellulose x-ray reflections[4]. The implication of this result is that for most of the materials listed in Table 1 21-WAXS will not generate a reliable value of cellulose crystallinity. This finding is contrary to the authors' earlier determination on cellulosic materials where they showed that 21-WAXS produced reliable values of crystallinity[1]. Therefore, for materials that contain hemicelluloses and/or lignin 18-WAXS (Segal-WAXS) is a better choice between the two x-ray methods.

Effect of Partial Delignification and Alk. H_2O_2 Bleaching on WAXS Crystallinities

To determine how this situation is impacted by partially removing lignin and upon alkaline peroxide bleaching (also called lignin-retaining bleaching) all the samples were subjected to the two treatments in separate stages. Conclusion was that for most of the samples the absolute values of 18- or Segal-WAXS crystallinity increased upon partial delignification (Table 2) and alk. peroxide bleaching. This, for the delignified samples, was in agreement with the expectation that reduced lignin's contribution in the cellulose diffraction region would lead to higher crystallinity (Table 2; increase was anywhere from 2 to 15%). Nevertheless, the difference between the 21-WAXS and 18-WAXS still remained quite large (not reported here). It's likely that additional removal of lignin and/or hemicellulose will lead to further reducing the difference between the two WAXS crystallinities.

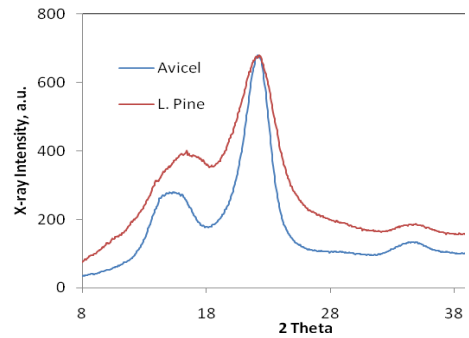

Fig. 1. WAXS diffractograms of avicel-PH-101 (bottom) and loblolly pine (top).

Table 2. 18- or Segal-WAXS crystallinities of extracted and chemically modified samples

Sample #	Extracted /%	Partially delignified/%	Alk. H_2O_2 bleached/%
Softwoods			
1	56.5	70.8	66.2
2	56.9	67.5	66.3
3	55.5	70.5	63.2
4	52.2	69.9	62.9
5	62.6	71.5	61.3
6	52.2	65.7	59.5
7	58.3	71.8	63.8
8	56.5	69.8	64.6
9	55.5	69.7	63.8
Hardwoods			
10	58.6	66.2	64.3
11	60.8	67.4	67.7
12	61.4	68.6	67.1
13	59.9	66.0	67.1
14	60.6	65.8	66.7
15	62.2	64.1	64.7
16	58.1	68.0	67.1
17	65.4	62.2	63.7
18	58.8	64.0	65.4
19	55.1	65.4	66.3
20	61.7	68.5	65.9
21	56.6	65.5	61.3
Ag residues			
22	56.7	59.7	63.5
23	59.3	65.8	65.0
24	65.7	70.2	70.1
25	56.3	60.9	63.7
26	66.1	69.3	60.3
27	51.1	58.4	59.3
28	78	83.4	83.6
29	86.6	88.6	88.5
30	44.5	60.1	58.7

Pulps			
31	76.5	77.9	77.7
32	70	79.6	76.8
33	70.4	79.5	75.7
34	64.2	67.5	66.7
35	62.6	63.5	59.2
Compression vs. opposite woods			
36	60.7	63.5	59.2
37	58.4	66.0	60.4
Heartwood vs. sapwood			
38	54.6	62.6	62.2
39	54	62.5	62.6
40	55.7	65.3	59.9
41	54.7	65.8	60.1

On the other hand, most samples where crystallinity increase was only little or absent were either low lignin samples (e.g., #**26**, #**29**, and #**31**). Nevertheless there were a few exceptions as well. Display of increased crystallinity was also the characteristic of the samples that were bleached with alk. peroxide. As of now there is no straight forward explanation for this observation because the bleaching is not expected to remove lignin and/or hemicelluloses.

Univariate Raman Crystallinities

Crystallinities determined using the two univariate-Raman methods are listed in Table 3. 21-univariate Raman refers to the method that was developed by the authors in 2010 (called univariate-Raman in [1]) and used the calibration set data that were derived from the 21-WAXS crystallinity for the control Whatman CC31 sample. Except for the 2 samples (flax and banana stem, #s **28** and **30**, respectively), Raman spectra of others were good enough for measuring the crystallinity. Nonetheless, the spectra of few samples (lodglepole pine - # **5**, eucalyptus - # **21**, and unbleached kraft pulp - # **31**) generated significantly higher values compared to not only the other similar samples but also the 18-WAXS crystallinities (Tables 2 and 3). This is likely to be due to the underlying fluorescence in the spectra. Of the 21-univariate Raman crystallinities (Table 3, column 2), 92% were within the crystallinity range 45 to 69%. When the 21-univariate Raman and 18-WAXS data were compared, the values were similar for a large number of samples. However, there were several samples whose values differed significantly. The samples where crystallinities differed by at least 10 points were aspen (# **11**, lower in Raman), kenaf core (# **25**, higher in Raman), sugarcane bagasse (# **27**, higher in Raman), bleached softwood (# **32**) and hardwood (# **33**) kraft pulps (both lower in Raman), white oak heartwood (# **38**) and sapwood (**39**; both higher in Raman), and eucalyptus (# **21**, higher in Raman). This is being investigated further.

Table 3. Univariate raman crystallinities of extracted samples

Sample #	21-Univariate Raman /%	18-Univariate Raman /%	% Lower Compared to 18-Univariate
Softwoods			
1	52.3	62.8	16.7
2	50.0	60.0	16.7
3	52.8	63.3	16.6
4	45.4	54.3	16.4
5	78.1[a]	94.1	17.0
6	51.6	61.9	16.6
7	49.0	58.7	16.5
8	47.1	56.5	16.6
9	53.0	63.5	16.5
Hardwoods			
10	53.0	63.6	16.7
11	50.8	60.8	16.4
12	63.6	76.4	16.8
13	51.0	61.1	16.5
14	58.6	70.3	16.6
15	67.6	81.3	16.9
16	61.0	73.3	16.8
17	57.7	69.3	16.7
18	63.5	76.3	16.8
19	58.7	70.4	16.6
20	56.1	67.4	16.8
21	73.9[a]	88.9	16.9
Ag residues			
22	51.6	61.8	16.5
23	64.9	78.1	16.9
24	64.9	78.0	16.8
25	68.7	82.7	16.9
26	66.6	80.1	16.9
27	62.2	74.7	16.7
28	—[b]	—[b]	—[b]
29	62.4	75.0	16.8
30	—[b]	—[b]	—[b]
Pulps			
31	83.9[a]	101.1[a]	17.0
32	48.0	57.5	16.5
33	47.5	56.8	16.4
34	64.6	77.6	16.8
35	60.7	73.0	16.9
Compression vs. opposite woods			
36	50.9	61.0	16.6
37	51.9	62.2	16.6
Heartwood vs. sapwood			
38	66.8	80.4	16.9
39	63.9	76.8	16.8
40	51.0	61.2	16.7
41	55.0	66.0	16.7

[a]Crystallinity value not reliable due to fluorescence in the spectrum.
[b]Raman spectrum of poor quality to calculate crystallinity

18-Univariate Raman Crystallinities

To address the issue of the importance of having a Raman calibration method based on the WAXS crystallinity at 2θ = 18° instead of 21°, calibration set crystallinities were derived that were based on the 18-WAXS data for the control Whatman CC31 sample. Subsequently, as was done in the publication [1], linear calibration regressions were developed between 380/1096 Raman intensity ratios and Segal-WAXS data vs. calibration set crystallinities (Fig. 2).

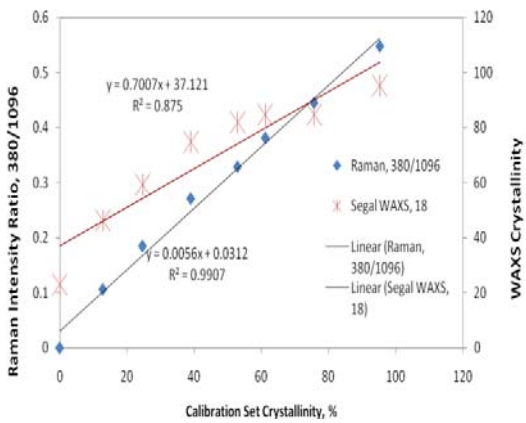

Fig. 2. Correlation between 380/1,096 Raman intensity ratios and 18-WAXS crystallinities against calibration set crystallinities

It is clear from Fig. 2 that compared to 18-WAXS Raman intensity ratio calibration (called 18-univariate Raman) is much better (R^2 = 0.88 vs. 0.99, respectively). Raman crystallinities in Table 3 (column 3) correspond to this calibration.

Comparing the two sets of Raman crystallinities in Table 3, it is clear that the 18-univariate method generated higher values (by about 17%). This originated from the fact that a 16%-higher crystallinity value, for the control Whatman CC31 sample, was used in the calculations of the crystallinities of the calibration set samples. Although either Raman method can be used to determine the crystallinity of the materials, for reasons that have to do with the contribution of the amorphous cellulose at 21° in the WAXS diffractogram, the authors recommend use of the 21-univariate Raman method[1].

Effect of Delig. and Alk. H_2O_2 Bleaching on Raman Crystallinities

In the Raman crystallinity measurement methods, there are two factors that can influence the data. The first one is the existence of lignin contribution and the other is the presence of sample fluorescence in the spectrum. As far as the role of the former is concerned, from past studies it is known that compared to softwood lignin hardwood lignin (or syringyl lignin) has a considerable contribution at 370 cm^{-1}[5]. This band can therefore influence the intensity of the neighboring 380 cm^{-1} peak when the crystallinities of the hardwood samples (or those samples that contain the syringyl lignin) are calculated. This implies that in the absence of other band intensity influencing factors if the lignin contribution at 370 cm^{-1} is removed, the crystallinity would decline (see Raman crystallinity equation in [1]). For example, that's what would be expected from the data on the hardwoods that were partially delignified (Table 4, sample #s **10** to **21**, **38**, and **39**). That was the case for most samples except in a few cases where the Raman crystallinity either increased slightly (American elm, # **10** and aspen # **11**) or declined too little (sour orange, # **17**). The latter seemed to have happened due to the presence of significant fluorescence contributions in their Raman spectra (not shown here).

On the contrary, softwood samples (Table 4, samples #s **1** to **9**, **36**, **37**, **40**, **41**) and mechanical pulps from such woods (# **34**, # **35**) produced no such decline in the crystallinity. In most cases, a small increase was detected which may have been a consequence of fluorescence reduction. Note that fluorescence, depending upon its contribution profile, can either increase or decrease the true Raman crystallinity of a sample. Moreover, because in most mature agriculture fibers lignin resembles hardwood lignin (lignin is syringyl type) the partial delignification of th samples in the Ag residues category resulted in reduced crystallinity (Table 4). The only exceptions where the value did not change much were alfalfa (# **22**) and ramie (# **29**). This may have to do with the small amounts of lignin in these two samples. Moreover, worth mentioning is also the fact that as the forages mature lignin's guaiacyl to syringyl ratio shifts towards syringyl type.

Table 4. Effect of partial delignification and alk. H_2O_2 bleaching treatments on 21-univariate-Raman crystallinities

Sample #	Extracted /%	Partially delignified /%	Alk. H_2O_2 treated /%
Softwoods			
1	52.3	56.1	59.9
2	50.0	49.5	63.3
3	52.8	54.0	54.2
4	45.4	49.6	51.0
5	78.1[a]	52.5	56.8
6	51.6	54.0	58.4
7	49.0	57.3	61.1
8	47.1	51.3	53.3
9	53.0	57.8	61.6
Hardwoods			
10	53.0	54.8	65.8
11	50.8	56.1[a]	54.7
12	63.6	55.6	66.8
13	51.0	45.5	57.3
14	58.6	46.5	63.9
15	67.6[a]	59.5	66.6

16	61.0	56.6	68.1
17	57.7	56.7	63.9
18	63.5	52.5	66.8
19	58.7	53.9	63.2
20	56.1	54.4	61.0
21	73.9[a]	54.7	63.6
Ag residues			
22	51.6	51.6	50.6
23	64.9	57.7	59.4
24	64.9	52.2	59.1
25	68.7	51.6	62.0
26	66.6	53.2	54.5
27	62.2	52.8	61.7
28	—[b]	60.1	—[b]
29	62.8	64.3	67.8
30	—[b]	47.9	—[b]
Pulps			
31	83.9[a]	51.8	67.5
32	48.0	57.4	57.9
33	47.5	58.2	54.6
34	64.6	56.7	71.8
35	60.7	60.9[a]	63.4
Compression vs. opposite woods			
36	50.9	53.1	51.6
37	51.9	56.6	52.7
Heartwood vs. sapwood			
38	66.8	58.0	66.9
39	63.9	57.7	61.8
40	51.0	54.5	59.6
41	55.0	55.6	55.3

[a]Treated with $NaBH_4$ after partial delignification to reduce fluorescence.
[b]Raman spectrum of poor quality to calculate crystallinity

The samples were bleached with alk. H_2O_2 to reduce the fluorescence contribution in the spectra. It's well known that such a treatment does not remove lignin and therefore, the comparison of the crystallinity data between the extracted and alk. H_2O_2 bleached samples should indicate how the presence of fluorescence influences the Raman crystallinity measurement. The foregoing is true as long as it is assumed that during the alk. H_2O_2 treatment the influence of any removed hemicelluloses is minimal and does not warrant inclusion.

Considering Table 4 data once again, for most woods that were bleached with alkaline H_2O_2 there was an increase in the crystallinity (Table 4, samples 1-20). On the other hand, most agriculture residues showed a decline (samples #s **22** to **30**). This decline may have to do with the possibility that in the Raman spectra of extracted samples the fluorescence contributions were present strongly and weakly in the 1096 and 380 cm^{-1} regions, respectively. In Raman spectroscopy, the strong fluorescence contribution in a region results in the reduction of the intensities of the Raman bands present in that region. Consequently, as this contribution was removed by bleaching, the 1096 cm^{-1} intensity was enhanced resulting in lower value of crystallinity (see Raman crystallinity equation in[1]).

CONCLUSIONS

Using the 21-univariate Raman method, cellulose crystallinities of most of the materials were accurately determined. For samples that contained high amounts of syringyl lignin, partial delignification was necessary to obtain correct values of the crystallinity. In general, the Raman values were in good agreement with the Segal-WAXS crystallinities of the extracted samples. Bleaching by alkaline H_2O_2 resulted in better quality spectra which in turn produced more correct Raman crystallinities. This study indicated that the 21-univariate Raman method can be used to reliably measure cellulose crystallinity of lignin containing materials.

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