

DETERMINATION OF CELLULOSE I CRYSTALLINITY BY FT-RAMAN SPECTROSCOPY

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ABSTRACT

Two new methods based on FT-Raman spectroscopy, one simple, based on band intensity ratio, and the other, using a partial least-squares (PLS) regression model, are proposed to determine cellulose I crystallinity. In the simple method, crystallinity in semicrystalline cellulose I samples was determined based on univariate regression that was first developed using the Raman band intensity ratio of the 380 and 1096 cm^{-1} bands. For calibration purposes, 90% crystalline Whatman CC31 and cellulose mixtures with crystallinities in the range 12-72% were used. When the intensity ratios were plotted against the theoretical crystallinities of the mixtures the plot showed a linear correlation (correlation coefficient $R^2 = 0.995$). Standard error calculated from five replicate Raman acquisitions indicated that the cellulose I Raman crystallinity model was reliable. Crystallinities of these cellulose mixtures were also calculated from x-ray diffractograms but these generated a correlation that was inferior compared to the one based on the Raman model. Additionally, using both Raman and x-ray techniques, sample crystallinities were determined from partially crystalline cellulose samples that were generated by grinding Whatman CC31 in a vibratory mill. The two techniques showed significant differences. In contrast to x-ray diffractograms, evidence of significant differences was present between the Raman spectra of low crystallinity celluloses and indicated that Raman spectroscopy was better suited to investigate differences between such samples.

In the second approach, a successful PLS (partial least squares) regression model for crystallinity, covering the 12-90% range, was generated from the same 7 calibration sample Raman spectra used in the simple method. The calibration model had a strong relationship between theoretical and predicted crystallinity values ($R^2 = 0.995$). The RMSEC (root mean square error of calibration) and RMSEP (root mean square error of prediction) values were 2.3 and 3.8%, respectively. RMSEP was the average prediction error, estimated in the validation stage and was subsequently validated with six independent ball milled Whatman CC31 samples. The regression coefficients obtained from the PLS model indicated that the cellulose bands, at 380 and 1096 cm^{-1} were the major contributors in building a robust model for predicting cellulose I crystallinity.

It was concluded that either of the two Raman methods could be used for cellulose I crystallinity determination in cellulose samples.

BACKGROUND

Cellulose crystallinity is defined as the mass fraction of crystalline domains in cellulose materials. This mass fraction can vary significantly in diverse materials. Crystallinity has an important effect on the physical, mechanical, and chemical properties of cellulose. For example, with increasing crystallinity, tensile strength, dimensional stability, and density increase while properties such as chemical reactivity and swelling decline. Some of the frequently used techniques for estimating cellulose crystallinity are wide-angle x-ray diffraction scattering (WAXS), solid state ^{13}C NMR spectroscopy, and FT-IR spectroscopy. Although WAXS is the most common technique, it involves isolation of amorphous background from the diffraction pattern which in case of cellulose crystallites is not always easy because the cellulose crystallites are small and for lower crystallinities, the spectra are poorly defined with weak broad features from which the contribution of the amorphous phase is difficult to estimate.

Raman spectroscopy has proven to be a useful technique in the cellulose and lignocellulose field with numerous applications [1, 2]. It has become an important analytical technique for nondestructive, qualitative, and quantitative analysis of cellulose containing materials. In particular, FT-Raman spectroscopy has the added advantage due to its ability to successfully analyze materials that are fluorescent in conventional Raman.

Crystallinity measurements using Raman on semicrystalline polymers including cellulose [3] have been performed. The principle behind Raman methodology is same as in FT-IR and NMR and has to do with using the spectral features whose intensity, bandwidth, and/or position are affected by crystallinity. To develop FT-Raman based cellulose I crystallinity quantitation method, Schenzel et al. [3] used the weak bands at 1462 and 1481 cm^{-1} (CH_2 bending modes) in conjunction with spectral deconvolution. However, considering that the intensities of the selected bands are quite low and that the process of deconvolution is not free of the band fitting problems, a better approach is desired. The objective of the present study was to develop another FT-Raman spectroscopy method so that cellulose I crystallinity in cellulose materials can be estimated accurately.

EXPERIMENTAL

Whatman CC31 powder was from Whatman plc, (Maidstone, UK). Lower crystallinity and amorphous cellulose samples were generated by grinding, for various durations, Whatman CC31 in a vibratory mill using steel balls. The milling was conducted in the cold room (5°C) for a prescribed time. The milling times were 2.5, 5, 10, 15, 30, 45, 60, 90, and 120 minutes. For calibration purposes, cellulose mixtures with crystallinities in the range 12-72% were produced using different mass fractions of 90% crystalline cellulose I Whatman CC31 and completely amorphous cellulose (120 min ball-milled Whatman CC31). This group of six samples (mixture 1 to

mixture 6, Table 1) along with the control Whatman CC31 was classified as calibration set.

FT-Raman

Cellulose samples were analyzed with a Bruker RFS 100 spectrometer (Bruker Instruments Inc., Billerica, MA). This Raman system is equipped with a 1064 nm 1000-mW continuous-wave (CW) diode pumped Nd:YAG laser. Samples were pressed into pellets. The laser power used for sample excitation was 600 mW, and 1024 scans were accumulated. Bruker's OPUS software program was used to find peak positions and process the spectral data. For plotting purposes, data were converted to ASCII format, then imported to Excel. Using OPUS, peak height was calculated by a baseline method that involved choosing a baseline point near the peak, drawing a horizontal line (from that point) under the peak, and choosing left and right edge points on the peak. This was done for both 380 and 1096 cm^{-1} peaks. The spectral data was exported to Excel where Raman intensity ratios were calculated and univariate regression models were developed.

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. The peak height of the peak present at about 22° corresponding to the 002 crystal planes was used for calculating the crystallinity index and determined from the ratio of the crystallinity part of the 002 peak to the total absolute peak height. The crystalline portion of the total contribution at 22° was calculated by the sloping background method which involved drawing a connecting a straight line between the 2θ values of 19° and 28° .

Multivariate Analysis

For multivariate data analysis, the Unscrambler 9.7 software program (CAMO Technologies, Woodbridge, NJ) was used. Calibration models were developed, using specific regions in the 250-1450 cm^{-1} range Raman data (Excel Raman files), with untreated and pre-processed spectra (normalized, 1st and 2nd derivatives). Principal component analysis (PCA) was used to reduce the original dimensions of the spectral data and represent the original data using new principal component (PC) scores that captured the maximum variation in the data. Using the first and second PC variables, PCA scatter plots were generated. The "leave-one-out" full cross validation technique was used to develop the PLS calibration model. The performance of the PLS models was assessed using several common statistical measures - the coefficient of determination, R^2 a measure of the strength of the fit to the data and RMSEC and RMSEP, measures of the calibration and prediction errors in the fit, respectively. Of the Whatman CC31 cellulose samples, seven were used for the PLS calibration model while 6 ball-milled samples were used for prediction.

RESULTS AND DISCUSSION

Univariate analysis

Raman spectra, in the region 250-1500 cm^{-1} , of selected ball milled Whatman CC31 are shown in Fig. 1 so that the spectral changes occurring upon loss of crystallinity can be visualized. Although a large number of bands were affected, the bands that experienced the most change are annotated. The intensity and bandwidth of two of these spectral features detected at 380 and 1096 cm^{-1} were significantly affected by crystallinity modification. Spectra of 30 and 120 min ball-milled Whatman CC31 [Fig. 1(c) and Fig. 1(d), respectively] were very similar indicating that milling durations greater than 30 min produced very small, if any, change in crystallinity.

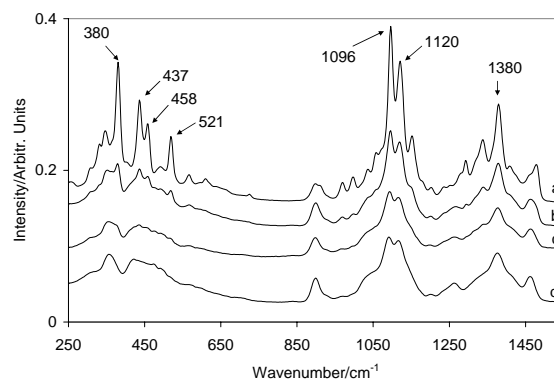


Figure 1: Effect of ball-milling (BM) on Whatman CC31 Raman spectra; (a) control, (b) 15 min BM, (c) 30 min BM, (d) 120 min BM

To determine if, using Raman spectroscopy, a value for the crystalline fraction can be obtained in Whatman CC31 samples that were ball-milled for various durations, a two-phase (crystalline and amorphous) model was used to calculate crystallinity over a wide range of compositions over at least 10%. Therefore cellulose crystallinity, X_c , can be defined and calculated as

$$X_c = M_c/M_t = M_c/(M_c + M_a)$$

where M_c , M_a , and M_t are the crystalline, amorphous, and total mass fractions of cellulose, respectively. The crystallinities of the calibration set samples (theoretical crystallinities) were calculated using the above equation and also by WAXS and Raman univariate methods (Table 1). In Raman, as in WAXS, an assumption was made that the spectrum is composed of two superimposed spectra arising from the amorphous and crystalline phases. The Raman spectra of the calibration set (cellulose mixtures and control with crystallinities in the range 12-90%) were obtained and the peak height ratios for various bands (380/1096, 380/2900, 437/1096, 437/2900, 458/1096, 458/2900, 521/1096, 521/2900, and 1098/2900) were calculated. Peak height ratios were then plotted against the theoretical crystallinities. The Raman ratio plot for 380/1096 (Fig. 2) generated excellent regression and showed good sensitivity to crystallinity change.

TABLE 1: CRYSTALLINITIES OF CALIBRATION SET SAMPLES – THEORETICAL, WAXS, AND RAMAN UNIVARIATE

Sample ID	Theoretical	WAXS	Raman Univariate
Control	90.3	90.3	90.8
Mixture 1	71.8	80.8	70.8
Mixture 2	57.6	74.1	57.2
Mixture 3	50.2	69.6	48.5
Mixture 4	37.0	63.5	38.0
Mixture 5	23.4	50.0	20.0
Mixture 6	12.2	38.0	14.8

Therefore the simple univariate method can be used to calculate cellulose I crystallinities in cellulose samples based on the following equation.

$$X_{\text{Raman}} = [(I_{380}/I_{1096}) - 0.1689]/(0.0046)$$

Additionally, for some calculated ratios where the intensity of the 2900 cm⁻¹ band was used, the correlation coefficients were also excellent but considering the fact that in FT-Raman the intensity of the 2900 cm⁻¹ band can be affected by the sample moisture, such cases were not favored. To determine if any additional features of the cellulose spectra could be useful in developing a crystallinity index FWHM (full width at half maximum) data were also correlated but the correlations were not good.

Reproducibility - Repeat Raman scans on the calibration set samples produced an average standard error of $\pm 1.1\%$.

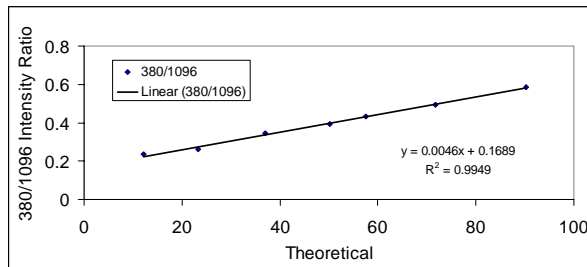


Figure 2: Univariate analysis of the calibration set Whatman CC31 samples showing the correlation between theoretical and 380/1096 Raman intensity ratio.

From the crystallinity data in Table 1, it is clear that the WAXS method overestimated the cellulose I crystallinity in those calibration set samples whose diffractograms have significant background (all except control sample in Table 1). As mentioned earlier, for lower crystallinity samples, the spectra are poorly defined with weak broad features from which the contribution of the crystallinity phase is difficult to estimate. The correlation coefficient for the WAXS versus theoretical

crystallinities was 0.9674, significantly lower compared to the Raman calibration.

Ball-Milled (BM) Samples Crystallinity Determination - The simple method based on the univariate analysis was used for the 2.5, 5, 10, 15, 30, 45, 60, 90 min ball-milled WhatmanCC31 I samples. These Raman crystallinities along with the WAXS and Raman PLS crystallinity data are listed in Table 2.

TABLE 2: CRYSTALLINITIES OF BALL-MILLED WHATMAN CC31 SAMPLES – RAMAN UNIVARIATE, WAXS, AND RAMAN MULTIVARIATE (PLS)

Milling Time min	Raman Univariate	WAXS	Raman PLS
2.5	68.6	83.5	70.0
5	49.8	78.6	52.3
10	37.8	68.3	36.2
15	31.5	54.6	28.4
30	18.2	32.8	21.1
45	15.8	33.6	18.3
60	16.8	21.6	—
90	19.7	26.7	—

Once again, WAXS method overestimated the cellulose I crystallinity in the ball milled samples because their diffractograms had significant background (all samples in Table 2). In Fig. 3, a regression curve between the Raman and WAXS methods showed an unsatisfactory correlation. As was the case with the calibration set samples, once again the empirical x-ray method was found to be inferior.

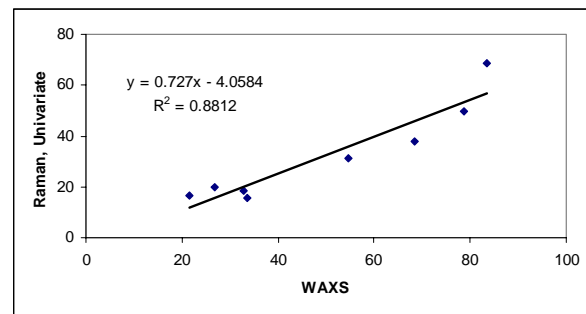


Figure 3: Regression curve between crystallinity estimates by Raman and WAXS for ball-milled Whatman CC31 samples.

Multivariate analysis

Having shown that univariate analysis produced a significant correlation for determining cellulose I crystallinity by Raman, it was of interest to establish whether multivariate data processing methods could be used as well.

Raman spectra in the region 250-450 cm^{-1} and 950-1200 cm^{-1} were used in the PCA and PLS analysis. These regions were selected because most useful changes occurred in these spectral regions. Although data were pre-processed in three different ways (normalized, 1st, and 2nd derivatives) and analyzed using Unscrambler, the best results were obtained for the normalized case. Therefore, in this section only results obtained using this form of pre-processed data will be discussed. PLS algorithm was run on the vector normalized data matrix, and the resulting Score and Loadings plots, were obtained. The purpose of the PCA, which is part of the PLS modeling, is to decompose the data matrix and concentrate the source of variability in the data into the first few principal components (PCs). Five PCs were chosen to calculate the model based on the calibration set but the calculation indicated that first 2 PCs were sufficient to describe the variation (Fig. 4). Although not shown here, the scatter plot of PC1 X PC2 indicated that 85 and 15% of the data variability was described by PC1 and PC2, respectively.

Score loadings for PC1 and PC2 are shown in Fig. 5. The loadings for both the PCs had strong positive correlations near 380, 430, 1096, and 1120 cm^{-1} . These wavenumber positions are similar to where changes due to cellulose crystallinity were most noticed in the original spectra (Fig. 1). The PLS model produced the RMSEC and RMSEP values of 2.3 and 3.8%, respectively. RMSEP is the average prediction error, estimated in the validation stage. As can be noted from Table 2 (column 4) and Fig. 6

the 6 independent ball-milled Whatman CC31 samples supported the 3.8% prediction error estimate.

CONCLUSIONS

Quantitation of cellulose I crystallinity in various Whatman CC31 samples was successfully carried out by either of the two methods developed in this work. The simple method which involved taking ratio of the intensity of two Raman bands was straightforward and reliable. In contrast, the 2nd method of calculating crystallinity was a PLS calibration model and involved coupling of FT-Raman data with chemometrics. However, the PLS method was also rapid, easy to use, and reliable.

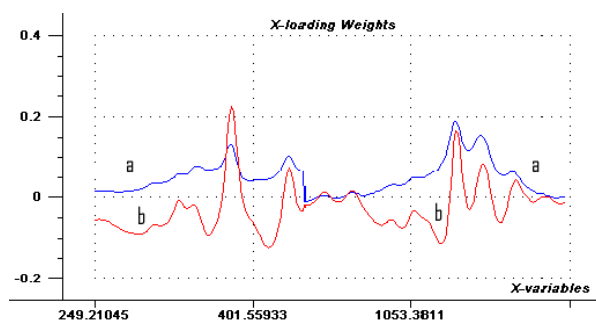


Figure 5: Score loadings with PC1 (a) and PC2 (b). The loadings have strong positive correlations near 380 and 1096 cm^{-1} .

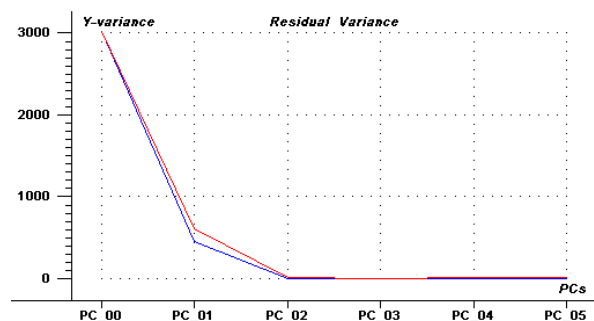


Figure 4: Residual variance decreased with increasing PC number indicating that crystallinity was related to Raman intensities.

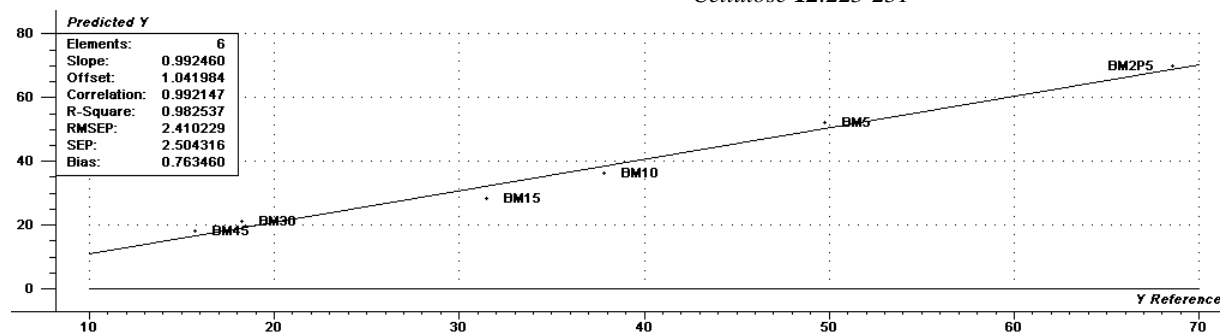


Figure 6: Correlation of the predicted and reference (Raman univariate) cellulose I crystallinities of the first 6 ball-milled samples listed in Table 2. BM2P5 to BM45 represent 2.5, 5, 10, 15, 30, and 45 minutes ball-milled samples.

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