FT-MID-IR SPECTROSCOPIC INVESTIGATION OF FIBER MATURITY AND CRYSTALLINITY AT SINGLE BOLL LEVEL AND A COMPARISON WITH XRD APPROACH

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Abstract

In previous study (Liu et al., 2011), we have reported the development of simple algorithms for determining fiber maturity and crystallinity from Fourier transform (FT) -mid-infrared (IR) measurement. Due to its micro-sampling feature, we were able to assess the fiber maturity and crystallinity at different portions of a single boll, as demonstrated here. Meanwhile, we attempted to compare the fiber maturity and crystallinity information between FT-mid-IR and X-ray diffraction (XRD) measurement. Despite considerable efforts in developing the curve-fitting protocol to evaluate the crystallinity index (CI) from XRD, in its present state XRD measurement can only provide a qualitative or semi-quantitative assessment of the amounts of crystalline or amorphous portion in a sample. The greatest barrier to establish quantitative XRD is the lack of appropriate cellulose standards needed to calibrate the measurements. In practice, samples with known CI are very difficult to be prepared or determined. As an approach, we might assign the samples with known CIs from FT-mid-IR procedure. Hence, this study also reported the development of a simple XRD algorithm, over time-consuming and subjective curve-fitting process, for direct determination of cotton cellulose CI by calibrating XRD with the use of CI_{IR} as references.

Introduction

Cotton fiber consists of natural cellulose I (β 1→4 linked glucose residues) and its quantity varies greatly with the stages of fiber growth (Hsieh, 2007; Abidi et al., 2010a & 2010b). Hydroxyl groups in cellulose are involved in a number of intra- and inter- molecular hydrogen bonds, which result in ordered crystalline and disordered (amorphous) regions. A parameter termed the crystallinity index (CI) has been used to describe the relative amount of crystalline portion in cellulose, based on the traditional two-phase cellulose model (crystalline vs. amorphous).

The crystalline structure of cotton fibers has been studied predominantly by X-ray diffraction (XRD) spectroscopy (Hsieh et al., 1997; Hu & Hsieh, 2001; Segal et al. 1962). To calculate the CI of cotton cellulose, two different methods have been used. The first one was developed by Segal and coworkers (1962), who estimated the CIs from simple formula of $100*(1 - I_{am}/I_{002})$ on the basis of two XRD peaks at $2\theta = 22.7^{o}$ and 18^{o}. As this method was proposed for empirical measurements to allow rapid comparison of cellulose samples, it should not be used as a method for calculating the amount of crystalline fraction in a sample (Park et al., 2010). One of most concerns is that the I_{am} value is significantly underestimated, resulting in an overestimation of the CI.

The second approach, also the most one being practiced, is spectral deconvolution method, in which individual crystalline peaks were extracted by a curve-fitting process from the diffraction intensity profiles (Hsieh et al., 1997; Hu & Hsieh, 2001; Jenkins & Snyder, 1996; Park et al., 2010). It requires computing software to separate amorphous and crystalline contributions to the XRD spectrum. During the curve-fitting protocol, a few assumptions have to be made, such as the shape and number of peaks as well as the deconvolution functions. Then, the CI is calculated from the ratio of the area of all crystalline peaks to the total area.

An important hypothesis for this analysis is that increased amorphous contribution is the main contributor to peak broadening. In addition to amorphous content, there are other intrinsic factors that influence peak broadening, such as crystallite size. It might be possible to deconvolute these contributions with well-behaved samples that can be resolved into many narrow diffraction peaks over a significant range of 2θ. Unfortunately, cellulose peaks are very broad, overlapped, and not well resolved. It is generally accepted in the cellulose community that peak broadening is due to the amorphous cellulose. Meanwhile, crystallite size is an equally important issue for peak broadening and information about average crystallite size has been assessed from XRD spectra (Hsieh et al., 1997; Hu & Hsieh, 2001). In order to reduce the effect of amorphous on CI readings, subtracting the amorphous contribution from XRD spectra using an amorphous standard was outlined by Ruland (1961). However, the challenge is to select an amorphous standard that is similar to the amorphous component in the sample. Traditionally, ball-milled cellulose has been utilized for such a purpose (Segal et al., 1962).

Other analytical tools, including solid-state ^{13}C nuclear magnetic resonance (NMR) (Larsson et al., 1997; Park et al., 2010), IR (Akerholm et al., 2004; Kataoka & Kondo, 1998) and Raman (Schenzel et al., 2005) spectroscopy, have been explored to investigate the structure and CI of diversified celluloses. However, there are few reports available that relate the CIs from XRD measurement to those from NMR, Raman or IR procedures. In a recent study, Park et al. (2010) compared the CIs of eight different celluloses between XRD and ^{13}C NMR, and found that the simplest XRD ratio method produced significantly higher crystallinity values than did the other methods.

In general, XRD determination of cellulose CI provides a qualitative or semi-quantitative evaluation of the amounts of either amorphous or crystalline components in a sample. Development of a reliable quantitative cellulose CI protocol is desired, by utilizing appropriate cellulose standards to calibrate or validate the XRD measurement. Apparently, these absolute standards are not easy to be prepared or determined.

In the latest FT-mid-IR investigation on immature and mature cotton fibers (Liu et al., 2011), we proposed a ratio algorithm R_2 by linking it with the relative amount of I_β to I_α crystal forms, represented by the respective IR bands at 708 and 730 cm^{-1}. Applying this algorithm to the data set consisted of 201 immature and 201 mature seed bolls; it suggested the R_2 range of 1.54 to 2.37 for immature fibers and 2.10 to 3.17 for mature samples. By setting an R_2 threshold value at 2.24, only 6 immature fibers and 10 mature samples were misclassified, yielding an overall 96% of correct identification accuracy. Despite of relatively weak intensities at 730 and 708 cm^{-1}, acceptable classification efficiency indicated the separation power of R_2 algorithm, which is anticipated because these values should be associated with cotton growth or development.

A need for rapid, accurate, and routine technologies to assess cotton crystallinity has been a subject of continuous interest for many years. Undoubtedly, the well-defined XRD method is mostly acceptable. However, its measurements are destructive (i.e., fibers to be cut or milled) and time-consuming (ca. 60 min/sample), and notably, the degree of CI depends on experimental parameters and subjective curve-fitting steps. This is one of many factors that it is difficult to compare the CIs from different research groups. For example, Sarna and Wlochowicz (2003) reported a CI range of 73 to 80% on Turkmenistan and Uzbekistan native cottons, which were higher than those of 53-69% on Egyptian cottons (Hindeleh, 1980), 57-64% on Saudi Arabian cottons (Khalifa et al., 1991), and 30-66% on upland cottons at growth stages of 21 to 60 days post-anthesis (DPAs) (Hsieh et al., 1997; Hu & Hsieh, 2001).

The objectives of this study were: (1) to verify the simple and universal ratio algorithms for quantitative determination of cotton CIs from FT-mid-IR measurement (CI_{IR}), (2) to propose a simple ratio algorithm for XRD analysis, (3) to correlate the XRD reading with known CI_{IR}, and (4) to convert the XRD reading into respective CI values (CI_{XRD}). Significantly, due to the micro-sampling feature of FT-mid-IR spectral acquisition, it is possible to look into the differences of both CI_{IR} and maturity between medium and tip portions within a locule, which is impossible through current measurements in cotton industry.

Materials and Methods

Seed Cottons

Seed and dried cotton locules (one of three to five segmented compartments in a boll) were collected from 18 cotton bolls at varying developmental stages of 21 through 60 DPAs. Three commercially available cultivars that represent a range in fiber micronaire were grown at Florence, SC, in 2010 crop year. The cultivars were chosen based on their average micronaire in the 2009 South Carolina Irrigated and Rainfed Official Variety Trials. The three cultivars are Deltapine (DP) 0949B2RF (average micronaire 4.8), Fibermax (FM) 1845LLB2 (average micronaire 4.45), and Phytogen (PHY) 565WR (average micronaire 4.15). Unlike the water rinsing of seed fibers described by Hu and Hsieh (2001), there was no any treatment on these bolls.

FT-Mid-IR Spectral Collection

All spectra were collected with an FTS 3000MX FT-IR spectrometer (Varian Instruments, Randolph, MA) equipped with a ceramic source, KBr beam splitter, and deuterated triglycine sulfate (DTGS) detector. The attenuated total reflection (ATR) sampling device utilized a DuraSamplIR single-pass diamond-coated internal reflection accessory (Smiths Detection, Danbury, CT), and a consistent contact pressure was applied by way of a stainless steel rod and an electronic load display. Ten measurements at one of two locations (either medium or tip positions) of one locule were collected over the range of 4000-600 cm^{-1} at 4 cm^{-1} and 32 co-added scans. Caution was taken to avoid both seed and visible trash portion in samples. All spectra were given in absorbance units and no ATR correction was applied. Following the import to Grams/AI software (V.7, Thermo Galactic, Salem, NH), the spectra were smoothed with a Savitzky-Golay function (polynomial = 2 and points =11). The data set was then loaded into Microsoft Excel 2000 to execute simple algorithm analysis.

XRD Spectral Acquisition

XRD data were obtained using a Rigaku Ultima IV Diffractometer. It was equipped with a copper anode sealed tube using an accelerating voltage of 40 kV with a tube current of 40 mA. The geniometer scanned a 2θ range between 5º and 40º with a scan rate of 0.7º/min. Ten cotton fiber samples from DP and PHY varieties were prepared by Wiley milling into a power that was passed through a 20-mesh screen. The cut fibers were placed on an aluminum hold (25 mm diameter x 2 mm deep) firmly and then mounted at the center of the goniometer circle for scanning. All spectra were imported to Grams/AI software and smoothed with a Savitzky-Golay function (polynomial = 2 and points =15), before exporting into Microsoft Excel 2000 to execute simple algorithm analysis.

Prior to FT-mid-IR and XRD measurement, all samples were well conditioned at a constant relative humidity of 65% and temperature of 22 ± 2 ºC for at least 48 hours.

Results and Discussion

FT-Mid-IR Maturity (M_{IR}) and Crystallinity Index (CI_{IR})

Figure 1 shows the representative FT-mid-IR spectra of developing cotton fibers at 20, 34 and 60 DPAs of DP variety in the 3600-600 cm^{-1} region. Band assignments for the cotton fibers have been studied in some detail (Hsieh, 2007; Abidi et al., 2010a; Liu et al., 2012). According to them, features between 3600 and 2750 cm^{-1} are from the O-H and C-H stretching vibrations. A broad band centered at 1640 cm^{-1} is attributed to the OH bending mode of water. Bands in the region of 1500-1200 cm^{-1} are mixtures of CH_2 deformations and C-O-H bending vibrations, and a number of bands in the 1200-900 cm^{-1} region are assignable to coupling modes of C-O and C-C vibrations, as well as the bands between 800 and 700 cm^{-1} are likely attributable to two crystal forms (I_α and I_β) of cotton cellulose.

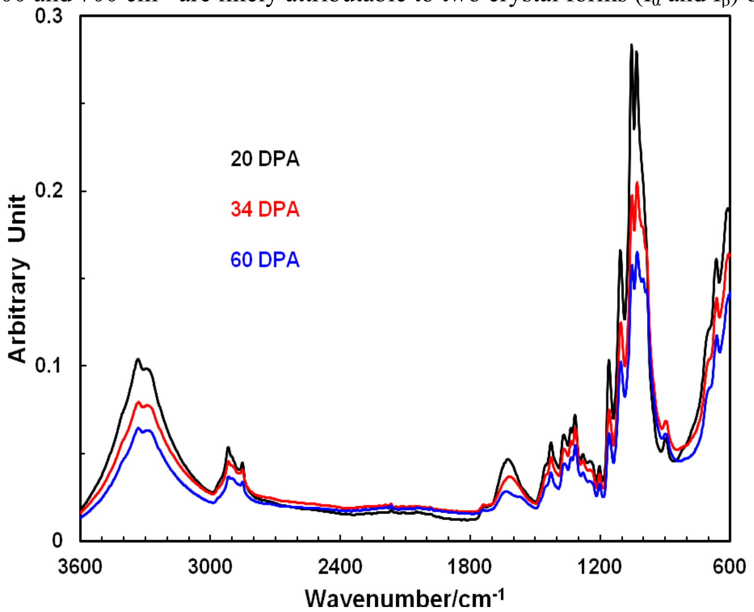

Figure 1. Representative FT-mid-IR spectra of cotton fibers at 20, 34, and 60 DPAs.

Comparison of the spectra reveals some common bands, indicating that the dominant FT-mid-IR bands arise from major common chemical component in cottons, cellulose. Given the significant structural and compositional variations among developing cottons, distinctive spectral differences occur in several wavenumbers, for example, pronounced shoulders at 2916, 1003, and 980 cm^{-1}.

In the previous study (Liu et al., 2011); on the basis of spectral distinctions between immature and mature fibers, we developed the 3-band ratio (R_1) in Eq. 1 and another one in Eq. 3 to assess the degree of cotton maturity (M_{IR}).

$$R_1 = (I_{956} - I_{1500}) / (I_{1032} - I_{1500}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (1)$$

$$M_{IR} = (R_1 - R_{1,\,sm}) / (R_{1,\,lr} - R_{1,\,sm}) \quad \ldots\ldots\ldots\ldots\ldots\ldots \ (3)$$

Eq. 3 was applied to the FT-mid-IR spectral set consisting of 18 locules (3 varieties x 6 DPAs), and resultant mean ± standard deviation (SD) of M_{IR} from 10 medium and 10 tip portions of respective locules are compared in Table 1. Given the limited number of cotton bolls, it is inappropriate to draw any solid conclusions about the three varieties, because fiber growth depends on environmental conditions, such as soil and climate. As expected, Table 1 reveals that M_{IR} increase from shorter to longer DPAs for all varieties, and relative to small change of M_{IR} between medium and end potions within some fibers, there were large variation among others.

Table 1. Comparison of M_{IR} for three cotton varieties at different DPAs.

Varieties	DP (Mean±SD)	PHY (Mean±SD)	FM (Mean±SD)
DPA = 20			
Medium	0.188 ± 0.026	0.374 ± 0.028	0.510 ± 0.150
Tip	0.192 ± 0.033	0.540 ± 0.044	0.211 ± 0.041
Average	0.190	0.457	0.360
DPA = 27			
Medium	0.591 ± 0.065	0.477 ± 0.059	0.444 ± 0.052
Tip	0.619 ± 0.067	0.578 ± 0.021	0.633 ± 0.113
Average	0.605	0.528	0.538
DPA = 34			
Medium	0.598 ± 0.144	0.626 ± 0.084	0.798 ± 0.039
Tip	0.781 ± 0.028	0.729 ± 0.074	0.479 ± 0.078
Average	0.690	0.678	0.638
DPA = 42			
Medium	0.733 ± 0.096	0.785 ± 0.077	0.739 ± 0.089
Tip	0.808 ± 0.050	0.834 ± 0.076	0.826 ± 0.101
Average	0.770	0.810	0.782
DPA = 53			
Medium	0.826 ± 0.085	0.807 ± 0.082	0.700 ± 0.128
Tip	0.778 ± 0.080	0.598 ± 0.157	0.558 ± 0.167
Average	0.802	0.703	0.629
DPA = 60			
Medium	0.849 ± 0.062	0.786 ± 0.106	0.751 ± 0.048
Tip	0.700 ± 0.082	0.840 ± 0.046	0.634 ± 0.115
Average	0.774	0.813	0.692

Applying a similar concept, we converted the R_2 values in Eq. 2 to CI_{IR} by Eq. 4.

$$R_2 = (I_{708} - I_{800}) / (I_{730} - I_{800}) \quad \ldots\ldots\ldots\ldots\ \ldots\ldots \ (2)$$

$$CI_{IR} = (R_2 - R_{2,\,sm}) / (R_{2,\,lr} - R_{2,\,sm}) \quad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

In which CI_{IR} represents for the CI through FT-mid-IR measurement, R_2, $R_{2,\,lr}$, and $R_{2,\,sm}$ are the R_2 value for unknown sample, the largest R_2 and the smallest R_2 values, respectively. If the most immature and mature fibers are assigned the CI_{IR} value of 0.0 and 100.0, respectively, the corresponding R_2 values should be the smallest and largest ones in Fig.3 (Liu et al., 2011). Hence, the $R_{2,sm}$ and $R_{2,lr}$ were determined to be 1.40 and 3.40, respectively, which were approximately 0.20 units smaller and larger than those observed in the Fig.3 (Liu et al., 2011).

Statistics of mean ± SD of CI_{IR} from medium and tip portion of various locules are summarized in Table 2. In general, CI_{IR} increases from shorter to longer DPAs for all varieties, and some fibers had larger differences between medium and tip potions than others.

Table 2. Comparison of CI_{IR} for three cotton varieties at different DPAs.

Varieties	DP (Mean±SD)	PHY (Mean±SD)	FM (Mean±SD)
DPA = 20			
Medium	27.68 ± 1.64	36.75 ± 1.10	42.99 ± 9.75
Tip	27.69 ± 3.47	30.37 ± 1.56	25.64 ± 3.60
Average	27.68	33.56	34.32
DPA = 27			
Medium	44.79 ± 5.61	43.42 ± 3.42	42.09 ± 2.85
Tip	44.48 ± 3.77	39.82 ± 4.10	52.79 ± 6.74
Average	44.63	41.62	47.44
DPA = 34			
Medium	52.36 ± 10.09	51.25 ± 9.67	68.10 ± 16.95
Tip	73.61 ± 8.55	55.90 ± 5.29	40.32 ± 5.00
Average	62.98	53.38	54.21
DPA = 42			
Medium	65.83 ± 10.43	73.37 ± 14.63	61.40 ± 7.58
Tip	66.60 ± 7.32	74.82 ± 4.03	72.04 ± 16.89
Average	65.92	74.10	66.73
DPA = 53			
Medium	77.53 ± 17.70	72.51 ± 11.56	64.18 ± 12.72
Tip	71.77 ± 19.49	49.86 ± 11.36	52.35 ± 11.35
Average	74.65	61.18	58.27
DPA = 60			
Medium	77.74 ± 12.68	79.05 ± 10.72	66.33 ± 8.82
Tip	62.28 ± 9.89	80.44 ± 11.49	54.22 ± 8.83
Average	70.01	79.75	60.28

Plot of M_{IR} vs. CI_{IR} values is depicted in Figure 2. Like the one of immature and mature fibers in earlier investigation (Liu et al., 2011), there is a clear separation among the fibers with differing DPAs. In general, both M_{IR} and CI_{IR} values increase with DPAs, which is reasonable because both indices should be sensitive to cotton growth. This observation is in well consistent with those from different approaches by other researchers (Hsieh, 2007; Abidi et al., 2010a, 2010b). For example, Abidi et al. (2010a) combined FT-IR and PCA to investigate the structural changes that occur during the different developmental stages, and concluded the existence of transition phase between the primary cell wall and the secondary cell wall around 17 and 18 DPAs within TX19 cultivar as well as 21 and 24 DPAs among TX55 cultivar. Meanwhile, through high performance liquid chromatography (HPLC) analysis of sugars content on these fibers, they (2010b) discovered the decreasing percentages of the sugars as the second cell wall developing, since the sugars are essential for cellulose synthesis allowing the formation of the secondary cell wall.

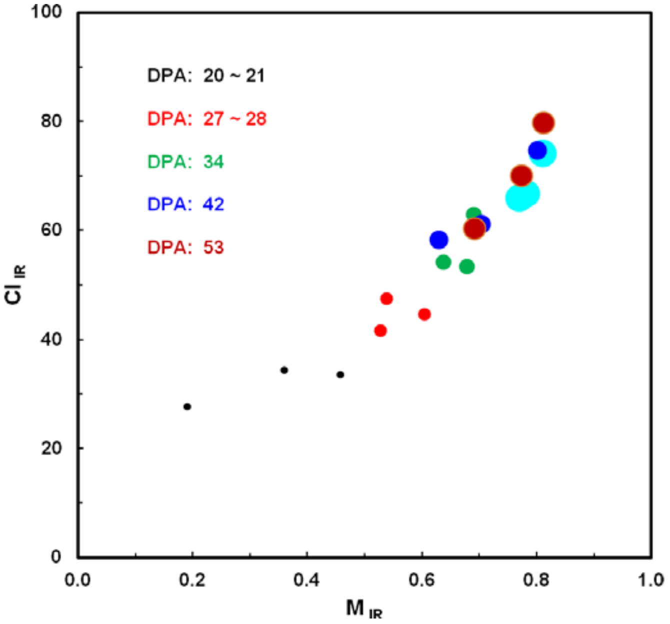

Figure 2. Plot M_{IR} vs. CI_{IR} values from a total of 18 locules, representing 3 varieties and 6 DPAs.

XRD Maturity (M_{XRD}) and Crystallinity Index (CI_{XRD})

The normalized and smoothed XRD spectra of identical fibers at developmental stages of 21, 34, and 60 DPAs are shown in Figure 3. The intact samples were scanned for FT-mid-IR spectra first, then they were cut into powders that were scanned for XRD spectra. The peaks located near two theta (2θ) = 14.9, 16.7, 22.8, and 34.6°, which are the characteristics of 101, 10$\underline{1}$, 002, and 040 reflections of cellulose I (Hsieh et al., 1997; Hu & Hsieh, 2001). With fiber growth, major peak intensity near 22.8° increases and its peak half-height width decreases, both of which suggested that the crystallinity and the crystallite dimension increase with secondary wall cellulose biosynthesis.

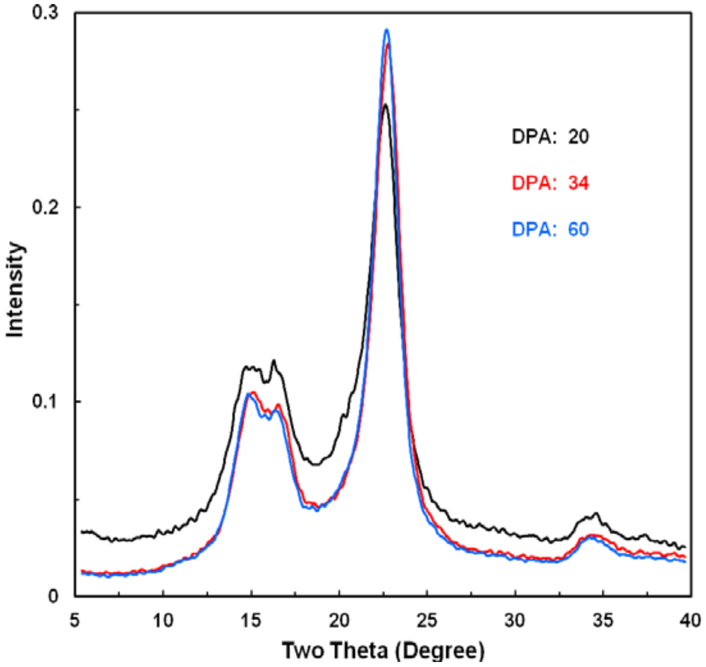

Figure 3. Typical XRD spectra of cotton fibers at 20, 34, and 60 DPAs.

Similarly, we proposed the 4-band ratio (R_3) in Eq. 5 and another 2-band ratio (R_4) in Eq. 6 to relate the XRD measurement with crystalline information.

$$R_3 = (I_{22.8} + I_{20.5}) / (I_{14.9} + I_{16.5}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R_4 = (I_{22.8}) / (I_{20.5}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where R_3 and R_4 are indicatives of crystalline information and $I_{14.9}$, $I_{16.5}$, $I_{20.5}$ and $I_{22.8}$ are each a three-point average of the intensity values at individual 2θ position.

As expected, both R_3 and R_4 values increase with DPAs (not shown), which is reasonable because two ratios should respond well to cotton development.

Relationship of CI_{IR} vs. CI_{XRD} and M_{IR} vs. M_{XRD}

In order to compare the crystallinity and maturity readings from FT-mid-IR and XRD measurement, Figure 4 and 5 show the plots of CI_{IR} and M_{IR} from FT-IR measurement against the R_3 and R_4 from XRD approach, respectively. Both regression lines revealed a higher correlation of $R^2 > 0.90$, which is within the expectation since two methods should be consistent in reflecting the compositional and structural changes among developmental cottons.

As a comparison, CI_{IR} was plotted against R_4 values that led to a R^2 of 0.87 whereas the pair of M_{IR} vs. R_3 generated a R^2 of 0.83 (not shown). Relatively lower correlations than those in Figure 4 and 5 might indicate the suitability of Figure 4 and 5 in characterizing the fiber crystallinity and maturity, respectively.

Furthermore, both R_3 and R_4 values could be converted into respective CI_{XRD} readings in the range of 0 to 100 and M_{XRD} index in the range of 0 to 1 by Eq. (5) and (6), as shown in a secondary vertical axis of both Figure 4 and 5.

$$CI_{XRD} = 100^*(R_3 – 1.317) / 0.91 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$M_{XRD} = (R_4 – 1.587) / 1.714 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

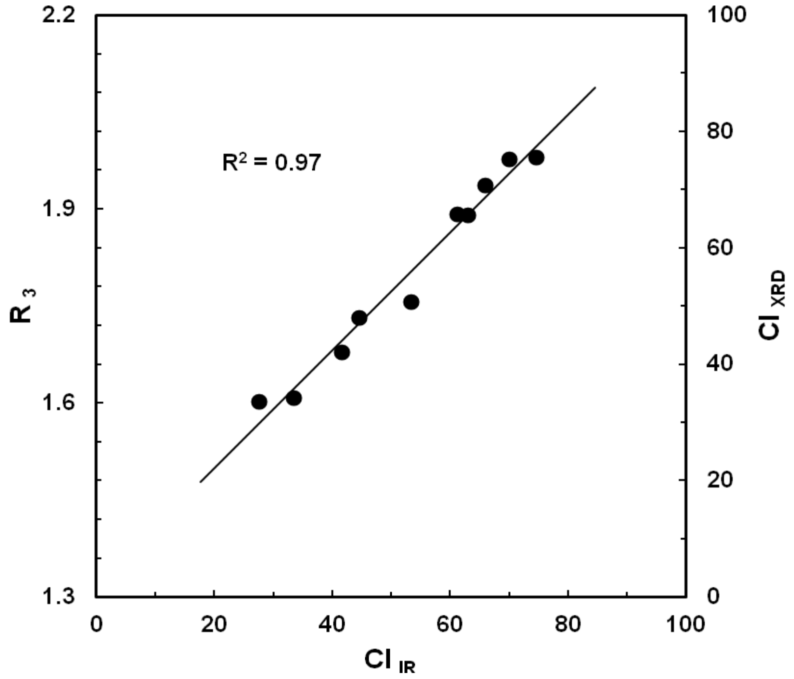

Figure 4. Relationship of CI_{IR} from FT-mid-IR procedure vs. CI_{XRD} from XRD measurements.

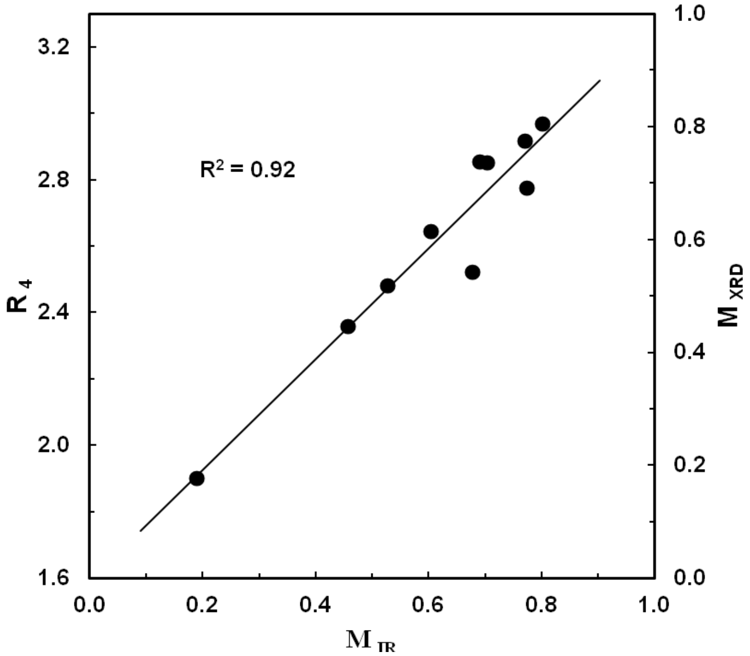

Figure 5. Relationship of M_{IR} from FT-mid-IR procedure vs. M_{XRD} from XRD measurement.

Table 3. Comparison of CI_{XRD} for diverse varieties from different groups

Varieties	DP [a]	PHY [a]	Maxxa[b]	Acala [c]	Turkmenistan / Uzbekistan [d]	Egyptian [e]	Saudi Arabian [f]
DPA = 20	31.2 (27.7)	31.9 (33.6)	31				
DPA = 21				30			
DPA = 25			54				
DPA = 27	45.6 (44.6)	39.7 (41.6)		45			
DPA = 30			64				
DPA = 34	63.0 (63.0)	48.2 (53.4)		55			
DPA = 36			66				
DPA = 42	68.0 (66.0)						
DPA = 48				56			
DPA = 53	72.9 (74.6)	63.2 (61.2)					
DPA = 60	72.4 (70.0)			58			
Others					73 - 80	53 - 69	57 - 64

[a] from this study; numbers in parenthesis were from FT-mid-IR measurements (CI_{IR}).
[b] Citied from Hu & Hsieh (2001).
[c] Citied from Hsieh et al. (1997).
[d] Citied from Sarna & Wlochowicz (2003).
[e] Citied from Hindeleh (1980).
[f] Citied from Khalifa et al. (1991).

Table 3 summarizes the CI_{XRD} for two varieties (DP and PHY) in this study. It ranges from 31.2% to 72.4% for DP cottons and 31.9% to 63.2% for PHY variety. Also, CI_{IR} values were outlined in parenthesis. In general, the CI_{XRD}

readings are in good agreement with Hu & Hsieh's values for developing cottons. Specifically, DP variety matches well with Maxxa cottons while the PHY fibers resemble Acala ones. When making a comparison with cottons from commercial bales, this observation are between the reported CI range of 73 to 80% (on Turkmenistan and Uzbekistan native cottons) and those of either 53-69% (on Egyptian cottons) or 57-64% (on Saudi Arabian cottons). Hence, it might suggest that the strategy reported here to assess cotton crystallinity is appropriate and reasonable.

Summary

This study validates the earlier development of FT-mid-IR spectroscopy in rapid, non-destructive, and direct determination of cotton fiber maturity and crystallinity. Given the micro-sampling feature in FT-mid-IR technique, it is likely to examine the differences of both CI_{IR} and M_{IR} characteristics at varying portions within a locule or a sample. As anticipated, both CI_{IR} and M_{IR} readings increase with fiber DPAs.

From XRD spectral intensity differences between different DPAs, major peaks were used to develop two simple ratio algorithms, which were then compared with the CI_{IR} and M_{IR} values. Strong correlations between CI_{IR} and R_3 (or CI_{XRD}) as well as between M_{IR} and R_4 (or M_{XRD}) suggested the equivalence and effectiveness of two separated measurements in crystallinity and maturity assessment. In other words, samples with easy- and fast- determinable CI_{IR} values could be used to calibrate the XRD measurement. Also, the CI from this study was in good consistent with those from traditional, time-consuming, and subjective protocol by other diverse researchers.

On the basis of practical implementation of this procedure for the determination of fiber maturity or crystallinity, this study described simple procedures to acquire the ATR device based FT-mid-IR spectra and to analyze the spectra. These procedures avoid the need to perform any pretreatments of cotton fibers, and also are advantageous in using small amount of fibers (as little as 0.5 mg) and in requiring only a short time (less than 2 min) for sample loading, spectral acquisition, and subsequent result report.

Disclaimer

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