

Chapter 4

Raman Spectroscopy in the Analysis of Cellulose Nanomaterials

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Cellulose nanomaterials (CNs) are new types of materials derived from celluloses and offer unique challenges and opportunities for Raman spectroscopic investigations. CNs can be classified into the categories of cellulose nanocrystals (CNCs, also known as cellulose whisker) and cellulose nanofibrils (CNFs, also known as nanofibrillated cellulose or NFCs) which when produced exist as aqueous suspensions at very low concentrations. Analysis of these suspensions by Raman spectroscopy is especially rewarding in that the CNs can be analyzed in the native hydrated state without special considerations. Several kinds of information can be derived from the spectra of the nanomaterials such as, estimation of crystallinity (in suspensions and freeze-dried states), measurement of accessibility of the nanomaterials by water, detection and quantitation of cellulose II polymorph in CNs, and effect of drying on the structure of CNs. Moreover, Raman spectra of the nanomaterials contain bands that are associated with chemical functionalities usually present on the surfaces of prepared/modified materials, for example, sulfate esters present on the surfaces of the sulfuric acid produced CNCs. In particular situations, these groups can be quantified by Raman spectroscopy. To accurately estimate crystallinity of CNs, a new method based on $\sim 93\text{ cm}^{-1}$ Raman band is reported. In contrast to the existing crystallinity estimation methods, the 93 cm^{-1} method has the capability to distinguish

between simply “organized” and “organized and crystalline” celluloses. The measurement of the latter fraction is necessary to accurately measure crystallinity. Finally, a few applications of Raman spectroscopy to CN-composites are considered and it is shown that useful information can be obtained from the Raman investigations.

Introduction

Cellulose materials have been analyzed by Raman spectroscopy since early 1970s (*1–20*). During the early stages, the main focus was on carrying out the band assignment work (*2*), studying orientation/organization of cellulose in materials (*2, 4, 6, 7*), and identifying bands that were characteristic of various cellulose polymorphs (*1*). In the context of the latter, Raman spectra of the celluloses are very sensitive to differences in conformation and therefore, helpful in identifying and distinguishing the various polymorphs of cellulose. Since then, a number of developments have furthered the practicality of Raman spectroscopy in the field of cellulose science. Some of these developments were technological in nature (*21*) (e.g., filters, detectors, lasers, and instrumentation) while the others had to do with interpretations of cellulose spectra (*2*) and Raman applications (e.g., estimating cellulose crystallinity (*16–18*), cellulose accessibility (*20*) etc.). As an example of instrumental advance, upon the development of Raman microprobe and confocal Raman imaging, not only specific micron and sub-micron size regions of a heterogeneous sample could be investigated (*2, 4*), but chemical mapping and therefore, distribution of components/polymers in a multicomponent sample could be studied (*6, 7*). This capability was particularly useful in the analysis of plant cell walls which are natural composites of cellulose, hemicellulose, and lignin. With the production of new CNCs incorporating composites, the same technique was used to investigate spatial distribution of CNCs in extruded composite filaments of CNCs and polypropylene (*22*) and in assessing the degree of mixing and the interface between polyethylene and CNCs (*23*). Additionally, development of FT Raman systems, using near IR excitations, has greatly enhanced the studies of biomass samples (*5*), most of which fluoresced excessively when only visible laser excitations were available.

Similarly, the topic of estimation of crystallinity of cellulose materials has benefited by the developments in the field of Raman spectroscopy (*16–18*). Currently, to estimate cellulose crystallinity, at least two methods exist which are both based on near-IR FT-Raman spectroscopy. Additionally, yet another method which is based on low frequency band at 93 cm^{-1} will be described for the first time in this Chapter. The earlier established Raman methods (*16, 17*) have been applied in numerous studies and are being increasingly adopted by the researchers in the cellulose materials field. Using the 380-Raman method (*17*) (also at times called Agarwal method), crystallinities of the CNCs were initially reported in 2010. Subsequently, the method was used to estimate crystallinities of a number of CNs (*24–26*).

Another recent advance has to do with the capability of Raman spectroscopy to measure accessibility of cellulose by water (20). Crystallinity and accessibility are not the same things and Raman spectroscopy provides means to measure both. The accessibility (A) measurement is based on the intensity increase of the band at 1380 cm^{-1} in a material's spectrum that was completely OH-to-OD exchanged by immersing in D_2O . More details on measuring "A" are provided later.

In this chapter, many of the above discussed applications will be described. The CNs used in the Raman spectroscopic work were derived, using well established methods, from various feedstocks (woods, pulps, cotton linters, bacterial cellulose, tunicin, and cladophora cellulose). Here, the CNs preparation methods are not described but the preparation methods are described in the appropriate references provided.

Cellulose Crystallinity

380-Raman Method

The 380-Raman method has been used before to estimate crystallinity of cellulose materials and the method has been described in detail (17, 18, 20). In the author's laboratory, a number of CNs (both CNCs and CNFs) were analyzed using the 380-Raman method (24–26). Such data is summarized in Table 1. Some of this data has not yet been published.

93-Raman Method

Another Raman method based on 93 cm^{-1} was developed for cellulose crystallinity estimation after it was noted that the intensity of this band was higher in higher crystallinity cellulose materials. The 93-Raman method has become possible because of the availability of 1064 nm-laser based FT-Raman systems. With the earlier dispersive visible-laser based Raman instruments, bands so close to the exciting line were overwhelmed by Rayleigh scattering. Identical to the approach taken earlier (17), for calibration purposes, 80.5% crystalline (estimated by Segal-method in ref. 17) and 120-min ball-milled (0% crystalline) Whatman CC31 and six cellulose mixtures (total 8 samples called calibration set) were produced with crystallinities in the range 10.9 – 64% (calculated based on the mass fraction of the crystalline portion in the calibration set samples) (17). Pellets were made and FT-Raman spectra obtained in the region 50 to 3700 cm^{-1} . Band intensity ratios of 93 cm^{-1} (peak height as well as peak area) and $1,096\text{ cm}^{-1}$ bands (peak height only) were plotted (Figure 1) against the crystallinities of the calibration set samples. The correlation plots (Figure 2) showed excellent linear correlations (peak height and peak area coefficients of determination $R^2 = 0.989$ and 0.988 , respectively).

Table 1. Crystallinities of CNCs and CNFs

Type of CN	Crystallinity		
	380-Raman		93-Raman
	CNCs		
	Freeze-dried	hydrated	Freeze-dried
HW kraft pulp	55.5	53.3	46.4
Heated poplar 200° C	62.0	58.0	48.6
Cotton	77.1	62.7 ^b	67.9
Bacterial	77.3	75.7	79.0
Tunicate	69.9	74.7	92.8
Cladophora	72.1	80.2	58.1
	CNFs ^{c, d}		
Bleached softwood kraft pulp fiber	40.4	ND ^e	ND
Refined pulp fiber	38	ND	ND
Refined and microfluidized fiber	39	ND	ND
Enzyme treated and refined fiber	44	ND	ND
Enzyme, refined, and microfluidized fiber	43	ND	ND
TEMPO treated, refined, and microfluidized fiber	25	ND	ND
Pulp CNFs	36.9	40.5	20.2

^a All CNCs were produced using the sulfuric acid method and were freeze-dried. ^b Hydrated post freeze drying. ^c Some of the data was published in reference (25). ^d Some of the data was published in reference (25). ^e ND not done.

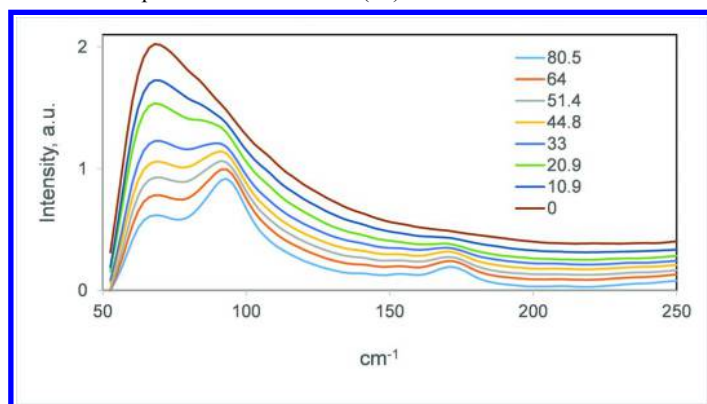


Figure 1. Normalized Raman spectra of the calibration set samples in region 50 – 250 cm⁻¹. Raman peak at 93 cm⁻¹ declines with the reduced crystallinity of the samples. (see color insert)

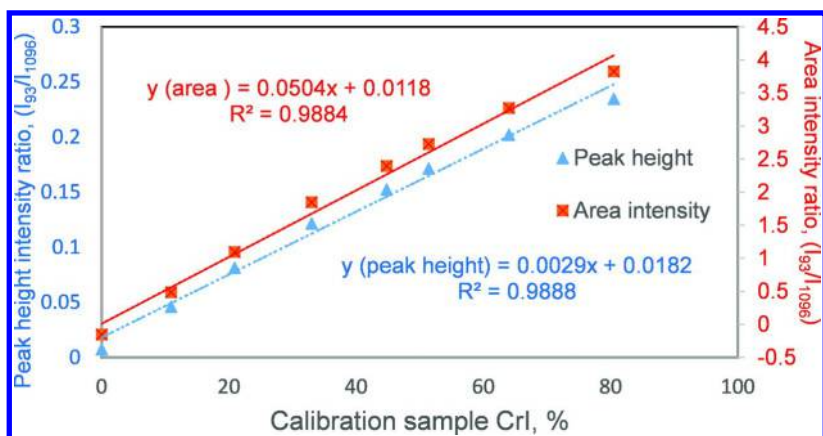


Figure 2. Correlation between Raman peak height Ratio (93/1096, primary y-axis) or Raman peak area Ratio (93/1096; secondary y-axis) and calibration sample crystallinity of the calibration set samples.

Using the peak height ratio correlation (Figure 2, Eq.1), the % crystallinities ($X_{93\text{-Raman}}$) of the CNs were determined (Table 1). Here, this method is referred to as “93-Raman”.

$$X_{93\text{-Raman}} = ((I_{93}/I_{1096}) - 0.0182)/0.0029 \quad (1)$$

For freeze-dried CNCs, when the 380-Raman and the 93-Raman crystallinity data were compared (Table 1), it became clear that the two methods generated significantly different values. Not only the absolute values were different, the crystallinity orders of the CNCs by the two methods were different as well. The reasons for this are understood at the moment to do with (a) I_{α} vs. I_{β} nature of the crystallites and (b) the characteristic that the 93-Raman is more reflective of cellulose crystallinity than the 380-Raman. With regard to (a), it was reported (19, 20) that in the Raman spectrum of *Valonia macrophysa* (highly crystalline I_{α} cellulose) the 93 cm^{-1} band was significantly less intense compared to tunicin (highly crystalline I_{β} cellulose). While other researchers have yet to report similar findings, this suggests that the 93-Raman method should be used to compare crystallinities within the family of I_{α} or I_{β} cellulose samples but not between the groupings. As far as justification for (b) is concerned, 93-Raman method estimated crystallinities of woods were 0 (author’s unpublished results) whereas those determined using the 380-Raman method gave values in the range 45 – 55% (18). The 93-Raman values supported the findings of Agarwal et al. who reported that the native state of wood cellulose is not crystalline (20). This implied that between the 380- and 93-Raman methods, the former measurement method was impacted by the organization of cellulose (cellulose in wood exists in organized state). However, that’s not the case for the 93-Raman method and therefore, the method is capable of making a distinction between “organized” and “organized and crystalline” celluloses.

In the hydrated state, except for tunicin and cladophora cellulose (both highly crystalline materials), for others, crystallinities were lower (Table 1). The latter

may have to do with the possibility that, upon drying, the supramolecular structure of cellulose in low crystallinity CNCs is modified and becomes less ordered. A less ordered cellulose will give a lower 1096 cm^{-1} band intensity (17), thereby increasing the estimated crystallinity by both the Raman methods (more below).

Never-Dried vs Freeze-Dried Pulp-CNCs

To evaluate the effect of freeze drying on the pulp-CNCs, spectra were obtained in four different ways: (a) in the never-dried suspended state, sampled in tube (Figure 3, never dried, in H_2O), (b) post freeze drying as a pellet (Figure 3, freeze-dried, pellet), (c) post freeze drying sampled in NMR tube (Figure 3, freeze-dried, in tube), and (d) freeze-dried then in H_2O , in NMR tube (Figure 3, freeze-dried then in H_2O). Spectral features are compared in the region $250 - 1850\text{ cm}^{-1}$ and are shown in Figure 3a and Figure 3b. To better visualize the spectral features, the spectra were plotted on expanded x-axis.

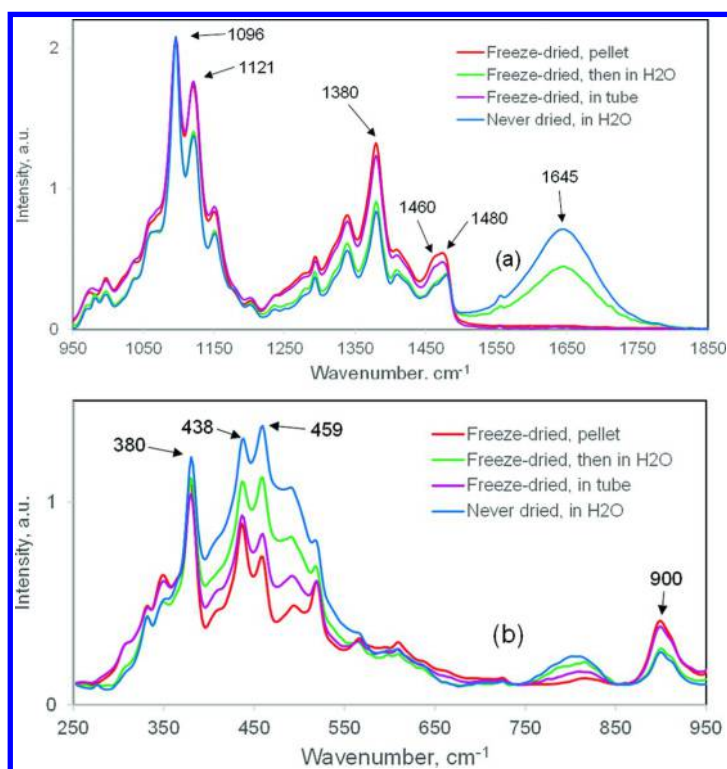


Figure 3. Raman spectra of pulp-CNCs in never dried, freeze-dried, and in water (post freeze drying) in region $950 - 1850\text{ cm}^{-1}$ (a) and in region $250 - 950\text{ cm}^{-1}$ (b). Freeze-dried CNCs were sampled as a pellet and also in an NMR tube. (see color insert)

Compared to the spectra of CNCs that were either never dried or suspended in water post freeze drying, the spectra of freeze-dried samples (either as pellet or in glass tube) had lower spectral resolution (Figure 3a, in the normalized spectra compare bands at 1096, 1121, and 1380 cm^{-1}). This suggested that freeze drying caused increased disorder in the CNCs. The spectral behavior was independent of how the CNCs were sampled (pellet vs. in tube, Figure 3a). Moreover, considering the $-\text{CH}_2$ bending region (1460 – 1480 cm^{-1}), drying resulted in an increased contribution at 1460 cm^{-1} . This suggested that some of the cellulose molecules present in *tg* conformation at C6 changed over to *gt* conformation upon drying (20).

A similar analysis of the Raman features in the lower frequency region, 250 – 950 cm^{-1} (Figure 3b), further supported the contention that CNCs became more disorganized upon freeze drying. The band at 900 cm^{-1} in the spectra of cellulose is known to be associated with increased disorder (17). As seen in Figure 3b, the two dry state spectra had higher intensity at 900 cm^{-1} compared to the wet states, once again indicating that the CNCs became less ordered upon drying. In the spectra of water suspended CNCs, the higher intensity seen in the 450 cm^{-1} region has to do with the increased contributions of the glass/ H_2O in this region. In absence of dried material in the tube, glass contributions are higher in the 450 cm^{-1} region and therefore, the intensities of 438 and 459 cm^{-1} bands cannot be directly compared in dry and wet states.

To more clearly visualize the spectral differences, normalized spectrum of the never dried CNCs was subtracted from the freeze-dried CNCs spectrum that was obtained in the tube. This way, the contributions of glass will be minimized in the subtracted spectrum because both CNCs were analyzed in tubes. The result is shown in Figure 4 (subtracted spectrum in black).

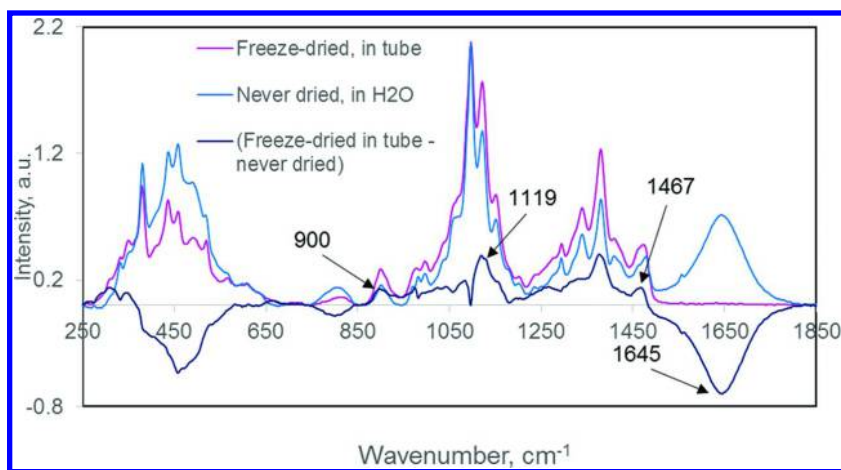


Figure 4. Raman spectra in the region 250 – 1850 cm^{-1} of pulp-CNCs in never dried and freeze-dried states that were sampled in NMR tubes. The subtracted spectrum (freeze-dried – never dried) is shown as well and some of its peaks are annotated. (see color insert)

Except for the negative broad features present at 470 and 1645 cm^{-1} which were due to glass and water, respectively, the prominent features in the subtracted spectrum were present in locations where contributions of disordered cellulose are expected (17). This substantiated the earlier stated result that these contributions were observed due to presence of disordered cellulose. It is difficult to say if the the disordered cellulose is inter-CNC in nature and therefore, only confined to the surfaces of the CNCs. The possibility of involvement of the interior chains (in a CNC) exists, especially in low crystallinity CNCs (Table 1, HW kraft pulp and heated poplar). In these CNCs, it's likely that at the tapering ends (where fewer cellulose molecules exist) of an individual CNC cellulose is not crystalline and hence, susceptible to water-induced reorganization. Moreover, the spectral changes in Figure 4 were not brought about by the process of pellet formation. This conclusion was based on the similarity of the spectra of CNCs-pellets that were prepared at different compressive loads.

Accessibility of CNs by Water

In 2016, Agarwal et al. (20) reported that in the Raman spectrum of a fully OH-to-OD exchanged sample of a material, the increase in the intensity of the band at 1380 cm^{-1} relative to 1096 cm^{-1} was a reflection of the non-crystalline nature of cellulose in that sample. This increase is due to the exchange of C6 OHs with ODs in the cellulose molecules. In Figure 5, a set of Raman spectra that depict this behavior is shown for a never dried cellulose sample (20) (never dried NaOH treated aspen wood holocellulose). Although more details are provided in Agarwal et al. (20), the never-dried sample was prepared by first delignifying aspen wood at room temperature for 5 weeks. After that, to remove hemicelluloses, the sample was treated by NaOH for 72 h at room temperature.

The measure of accessibility to water (A) is based on this increase of intensity at 1380 cm^{-1} and is defined by Eq. 2. "A" for amorphous cellulose is assumed to be 100%.

$$A_{\text{accessibility}} = \Delta I_{\text{sample}} * 100 / \Delta I_{\text{amorphous}} \quad (2)$$

where ΔI_{sample} is the % increase in the relative intensity of the 1380 cm^{-1} band in the Raman spectrum of the fully OH-to-OD exchanged sample and $\Delta I_{\text{amorphous}}$ is the % relative intensity increase for a similarly exchanged amorphous cellulose sample. For pulp-CNCs and amorphous cellulose, Agarwal et al. reported ΔI values to be 38.9 and 154.3 %, respectively (20). From Eq. 2, this gives an accessibility value of 25 (Table 2). That means only 25% of the cellulose is accessible to water. Accessibility data of pulp-CNFs is listed in Table 2 as well and, as expected, is significantly higher compared to CNCs.

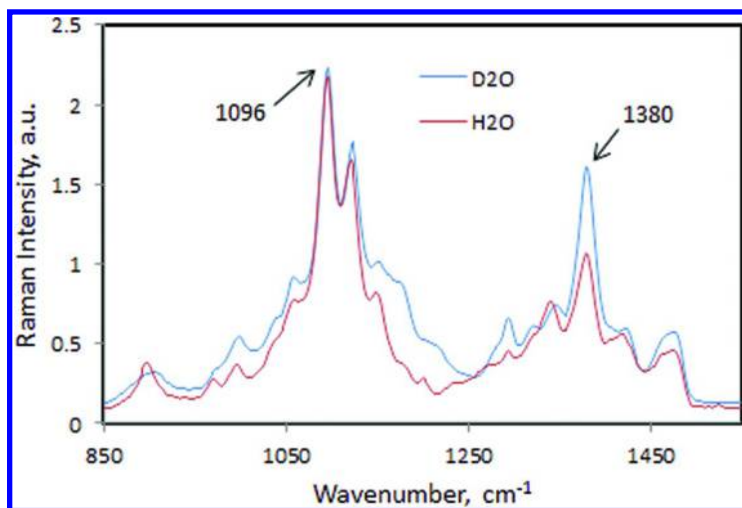


Figure 5. Increase in 1380 cm^{-1} relative intensity (relative to 1096 cm^{-1} , I_{1380}/I_{1096}) in a fully OH-to-OD exchanged sample of cellulose (never dried NaOH treated aspen wood holocellulose). Reproduced from (20). Copyright (2016, Springer). (see color insert)

Table 2. Accessibility data

CNs	Accessibility, %
Pulp-CNCs	25.0
Pulp-CNFs	48.6

Detection and Quantitation of Cellulose II in Pulp-CNs

When dissolving pulps are used as feedstocks in the production of CNs, pulp cellulose is not only present as cellulose I but some amount also exists as cellulose II due to the extra alkali stage involved. Therefore, one needs to detect and quantify the amount of cellulose II in the CNCs and CNFs that are derived from dissolving pulps. Raman spectroscopy can help by first detecting the presence of cellulose II and subsequently, based on the correlation shown in Figure 6, quantitating the cellulose II fraction.

Using FT-Raman spectroscopy, a cellulose II quantitation method was developed (not yet published) by mixing known quantities of cellulose I (Avicel PH-101) and cellulose II (mercerized Avicel PH-101). In addition to the pure cellulose I and cellulose II samples, five samples that were mixtures of cellulose I and cellulose II were used as a calibration set and their Raman spectra were obtained. A Raman intensity ratio (I_{577}/I_{1096}) was calculated by taking ratios of peak intensities at 577 (cellulose II band) and 1096 cm^{-1} (band due to both cellulose I and cellulose II). The ratio was plotted against the % cellulose II

present in the seven calibration set samples and the band intensity ratio was found to be linearly correlated with the amount of cellulose II (Figure 6, $R^2 = 0.9944$).

Based on this correlation (Eq. 3), cellulose II amounts were determined in two types of pulp-CNCs (Table 3) that were produced at different research laboratories. The cellulose II contents were significantly different although similar H_2SO_4 hydrolysis protocols were used in the production of the CNCs. This suggested that perhaps the feedstocks (dissolving pulps) had significantly varying amounts of cellulose II. Compared to the CNCs-064, the cellulose II content of the softwood dissolving pulp (for CNCs production) was higher (Table 3) – the pulp was used as a feedstock to produce the CNCs. This indicated that compared to cellulose I the cellulose II polymorph was slightly more susceptible to the acid hydrolysis.

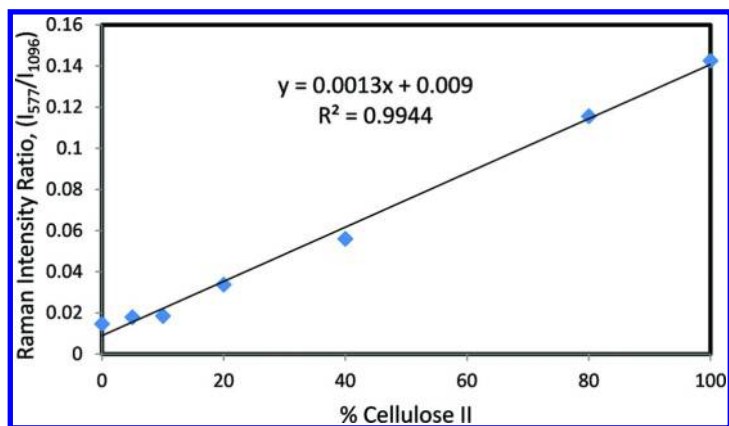


Figure 6. Correlation between Raman intensity ratio (I_{577}/I_{1096}) and cellulose II content of the calibration set samples.

$$\% \text{ Cellulose II} = ((I_{577}/I_{1096}) - 0.009)/0.0003 \quad (3)$$

Table 3. Quantitation of cellulose II in dissolving pulp-CNCs

Sample	Cellulose II, %
CNCs-048	17.8
CNCs-064	40.6
Dissolving pulp for CNCs-064	46.0

Detection of Sulfate Esters on CNCs-Surfaces

In Raman spectroscopy, a band due to sulfate esters groups is detected near 1060 cm^{-1} (27). In sulfuric acid extracted CNCs such groups are present on the surfaces of the nanoparticles and are generated by esterification of the hydroxyl groups resulting in the acid half ester $-OSO_3H$ or the sodium salt of the acid

half ester $-\text{OSO}_3\text{Na}$, if neutralized with NaOH . This results in the presence of negatively charged cellulose sulfate groups on the surface of the CNCs. In Figure 7, the spectra of CNCs that were sulfated to various degrees are shown. It was noted that, in the sulfuric acid hydrolyzed CNCs, the sulfation level was low (1 – 2%, based on Inductively Coupled Plasma Optical Emission Spectroscopy or ICP OES method) and the corresponding Raman band due to the sodium salt of the acid sulfate half ester at 1060 cm^{-1} was weak (Figure 7). However, to increase their sulfation level (3.7 to 7.0 %), when these CNCs were further treated by chlorosulfonic acid (28), this band became intense (Figure 7). These observations imply that at higher levels of sulfation, the sulfate groups can be detected and therefore, quantitated by Raman spectroscopy.

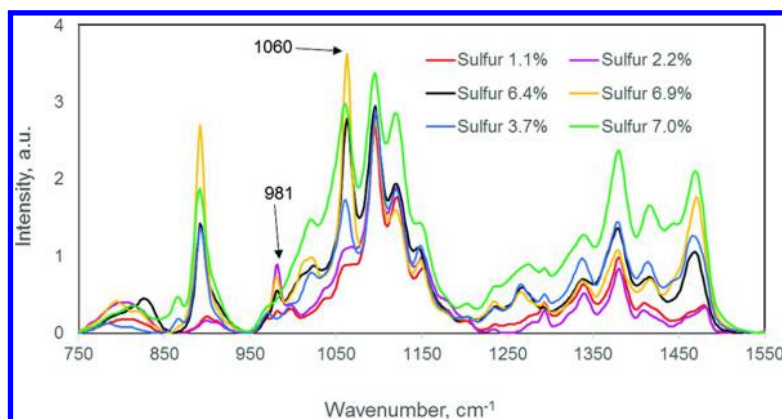


Figure 7. Raman spectra of sulfated CNCs. Sulfur content varied from 1.1 to 7.0%. (see color insert)

In the spectra shown in Figure 7, another non-cellulose band was detected at 981 cm^{-1} . This band is likely to be due to the NaSO_4 present in the CNCs because sulfate groups are known to contribute strongly at 980 cm^{-1} and a residual amount of NaSO_4 is expected in the CNCs even though they were thoroughly washed.

Studies of CNs in Composites

Raman spectroscopic techniques are being increasingly applied to investigate CN composites. Most often, such materials consist of CNs and synthetic polymers but composites between CNCs and CNFs have also been produced and investigated (author's unpublished results)

To evaluate dispersion of the CNCs at the microscopic level, investigation of a number of CNC-polypropylene (CNC-PP) composites by confocal Raman spectroscopy has been carried out (23). Such analysis showed that CNCs were aggregated to varying degrees in the composites and remained poorly dispersed in the polypropylene matrix. In Figure 8 a few Raman images of the composite fiber cross-sections are shown where distributions of PP (Figure 8a) and CNCs (Figure 8b – 8d) are shown. For further details on this study, readers are directed to publication by Agarwal et al. (22)

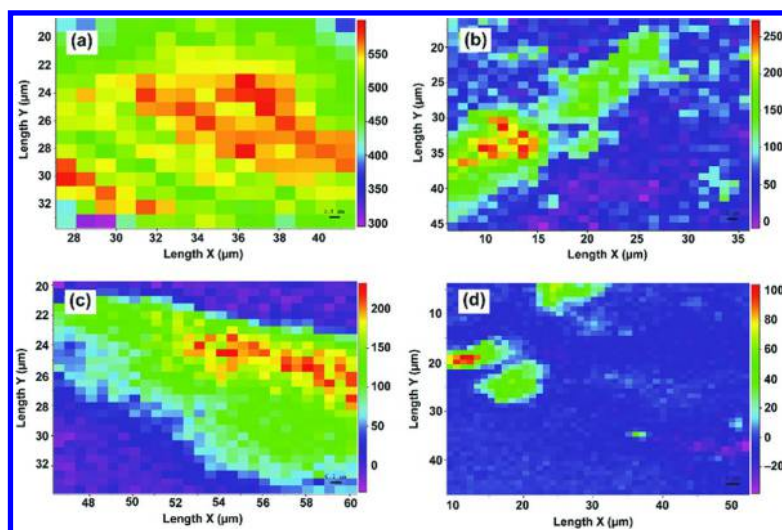


Figure 8. Raman images (false color) of spatial distributions of composite components: (a) PP in the PP control sample, (b) CNCs in the acetylated CNC and PP composite, (c) CNCs in a sample containing the nanosize fraction of microcrystalline cellulose and PP, (d) CNCs in sample of maleated PP, CNC, and PP. Note the different scale bar values in the different images ($a = 0.5\mu\text{m}$, $b = 1\mu\text{m}$, $c = 0.5\mu\text{m}$, $d = 2\mu\text{m}$). Reproduced from (22). Copyright (2012, Sage Publications). (see color insert)

Another important need in CN composite research is to understand how stress from matrix is transferred to CNs. Raman spectroscopy is able to provide such information. It has been reported that under mechanical tension the cellulose Raman band at 1095 cm^{-1} shifts (8). This characteristic of the cellulose band has been used by researchers to study what influences the stress transfer in various composites. For example, Rusli et al. (29) investigated tunicate CNC and poly(vinyl acetate) nanocomposites by polarized Raman spectroscopy and showed that the stress transfer was influenced by local orientation of the nanocrystals. Shifts of the 1095 cm^{-1} band as a result of uniaxial deformation of nanocomposite films were used to determine the degrees of stress experienced by the CNCs, not only due to stress transfer from the matrix to the tunicate CNCs but also between the CNCs within the composite.

A structure that used short cellulose nanofibrils (SCNF) as reinforcement in polyvinyl alcohol (PVA) fiber was also analyzed by Raman spectroscopy (30). Composite fibers were produced by adding SCNFs to PVA and spinning them into fibers. The molecular orientation of PVA was affected by a combination of wet drawing during gel spinning and hot drawing at a high temperature after drying. Peng et al. (30) reported that the strength and modulus of PVA/SCNF composite fiber with a SCNF weight ratio of 6 were nearly 60 and 220% higher, respectively than that of PVA by itself. As shown in Figure 9, shifts in the Raman peak at 1095 cm^{-1} indicated good stress transfer between the SCNF and the PVA matrix due

to strong interfacial hydrogen bonding and were responsible for increases in the strength and modulus of the fibers.

It is clear from these examples that, in a CN composite, a Raman shift in the 1095 cm^{-1} band can serve as sensor to detect the elastic stress or strain in the polymer matrix and reinforcement. Such information may be useful not only to evaluate distribution of stress but also to detect or predict the onset of failure in composite materials.

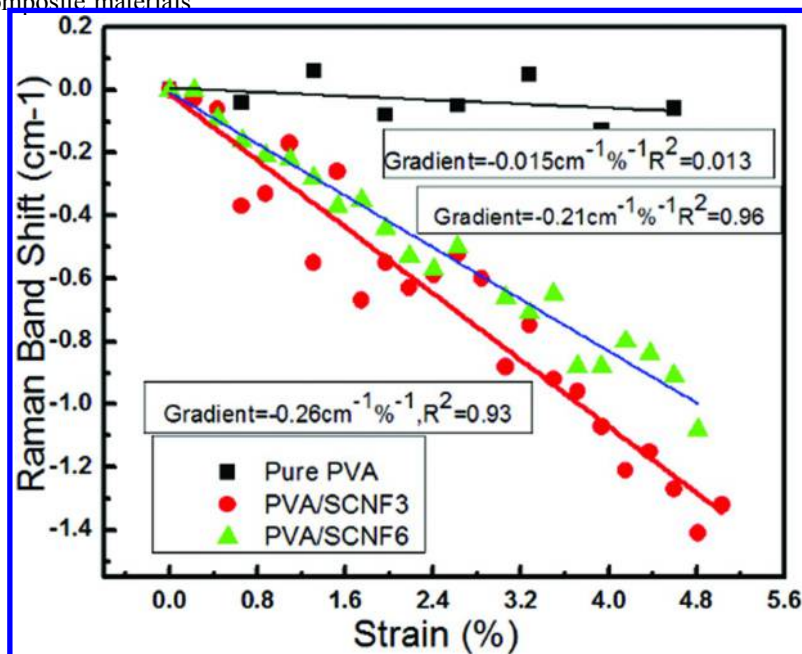


Figure 9. Shifts in 1095 cm^{-1} Raman band upon tensile deformation for pure PVA, PVA/SCNF3, and PVA/SCNF6 drawn fiber with a draw ratio of 27. Solid lines are linear regressions for the data. Reproduced from (30). Copyright (2014, Springer).

Conclusions

An overview of how Raman spectroscopy is being used to investigate CNs and CN composites was provided. It was shown that Raman methods can be used to estimate crystallinity of CNs in both never-dried and freeze-dried states. A brief summary of a new crystallinity estimation method based on 93 cm^{-1} band was provided. To measure accessibility of cellulose nanomaterials, the 1380 cm^{-1} band based Raman method was described. In cases where a CN consisted of both cellulose I and cellulose II polymorphs, it was shown how to detect and quantify the cellulose II fraction by Raman spectroscopy. The band at 1060 cm^{-1} was used to detect sulfate esters on the surfaces of CNCs. In case of CN composites, a couple of examples were described to demonstrate how Raman techniques are providing useful information on dispersion of the CN component and also on local stresses and strains in cellulose composite materials.

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