# Comparison of Cellulose Supramolecular Structures Between Nanocrystals of Different Origins

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## ABSTRACT

In this study, morphologies and supramolecular structures of CNCs from wood-pulp, cotton, bacteria, tunicate, and cladophora were investigated. TEM was used to study the morphological aspects of the nanocrystals whereas Raman spectroscopy provided information on the cellulose molecular structure and its organization within a CNC. Dimensional differences between the CNCs were found to be significant between the groups of tunicate, bacterial, & cladophora and pulp & cotton CNCs. The former group had the highest aspect ratio whereas the latter the smallest. Based on 380-Raman, the crystallinities of CNCs of different origins were different. The order of crystallinity for the CNCs, from high to low, was cotton = bacterial > cladophora > tunicate > pulp. Another property, water accessibility at the molecular level, was measured using a recently developed Raman method and the results indicated that compared to low accessibilities of tunicate and algal celluloses the accessibility of pulp-CNCs was significantly higher. Additionally, Raman information showed that, between the CNCs, differences between the $CH_2OH$ conformations were present.

## KEYWORDS

Bacterial cellulose, Cladophora cellulose, Cotton, Nanocrystals, Raman spectroscopy, Tunicate cellulose, Pulp

## INTRODUCTION

Nanocelluloses are the new exciting materials in the field of cellulose science. Their unique properties are a reflection of their nano-scale dimensions. Nanocelluloses can be divided into two classes –they are either cellulose nanofibrils (CNFs) or nanocrystals (CNCs). This study focuses on CNCs that were obtained from various celluloses. Some of the properties of the CNCs are that they have high mechanical strength, are transparent, have high surface area, and are birefringent. Moreover, due to the availability of high surface area, CNCs' surface can be modified to produce novel biomaterials that possess desirable functional properties. For example, it has been reported that poly(vinyl alcohol) and CNCs composites showed enhanced tensile storage modulus and the enhancement was dependent upon the type of the CNCs used.[1]

Considering that properties of cellulose nanocrystals (CNCs) depend upon their supramolecular structures, it is important to understand the structures in detail. In a CNC, although the supramolecular state of cellulose is highly organized, it remains adapted in reference to the original aggregated state from which a CNC is derived. While in each case, H-bonds and weak Van der Walls forces are the primary determinants of the structure, the assembled supramolecular state has contributions from a number of variables. Among others, these consist of local conformation of the $CH_2OH$ group, arrangement of the cellulose chains, and the types of intra- and inter-chain H-bonds.

CNCs of various kinds have been analyzed by TEM and AFM. Based on the literature reports,[2-4] the morphological information on the CNCs of interest is summarized in Table 1. With regards to the data in Table 1, one needs to remember that some types of CNCs can remain aggregated when dried from even most diluted suspension and therefore, the size (and therefore the aspect ratio) measurements can lead to some error.

**Table 1: Dimensions of the CNCs[a]**

| CNC Sample | Avg. length, nm | Avg. width, nm |
|---|---|---|
| Kraft pulp | 120 - 200 | 5 – 10 |
| Cotton | 150 | 15 |
| Bacterial | 800 - 1700 | 20 - 30 |
| Tunicate | 1600 | 40 - 50 |
| Cladophora | 1000 - 1500 | 20 |

[a]Based on literature [2-4]

In this investigation, using 64% $H_2SO_4$, CNCs were produced from kraft pulp & cotton and from celluloses from bacteria, tunicate, & cladophora. The resultant CNCs were characterized using a variety of methods that included TEM, IR, Raman, and XRD. The objective was to develop information on the molecular-structural aspects of the CNCs. In this abstract, most of the information presented is based on TEM and Raman spectroscopy.

## RESULTS AND DISCUSSION

### Morphology

Transmission electron micrographs (TEM) of the CNCs were obtained and are shown in Figs. la – le. The images were acquired using a Philips CM-100 TEM operated at 100kv. Although images were taken at different magnifications, only one TEM image of each of the samples taken at highest magnification is shown. Based on the TEM images, it was concluded that significant differences existed between the sizes and therefore, the aspect ratios of the CNCs. A more detailed analysis is currently underway which will provide more specific information.

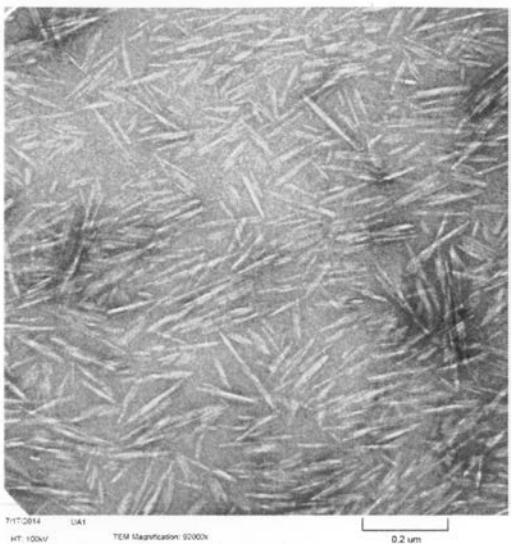

**Figure 1a. TEM of pulp CNCs.**

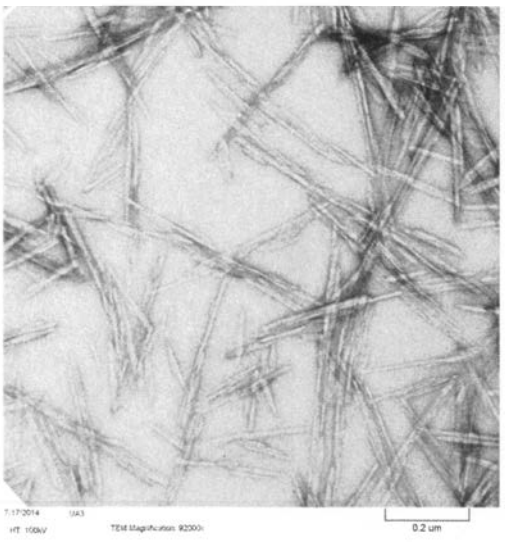

**Figure 1c. TEM of bacterial CNCs.**

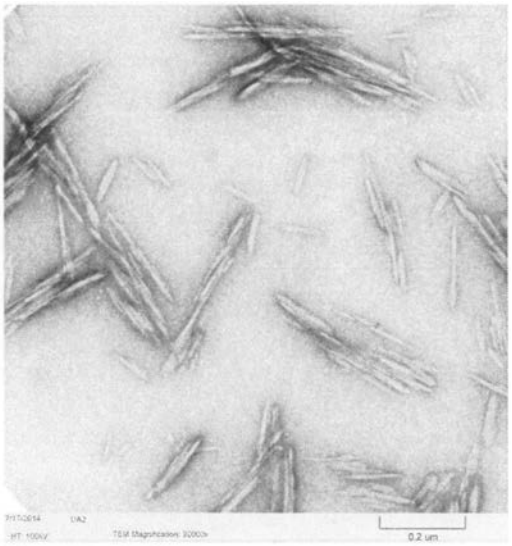

**Figure 1b. TEM of cotton CNCs; from WhatmanCC31.**

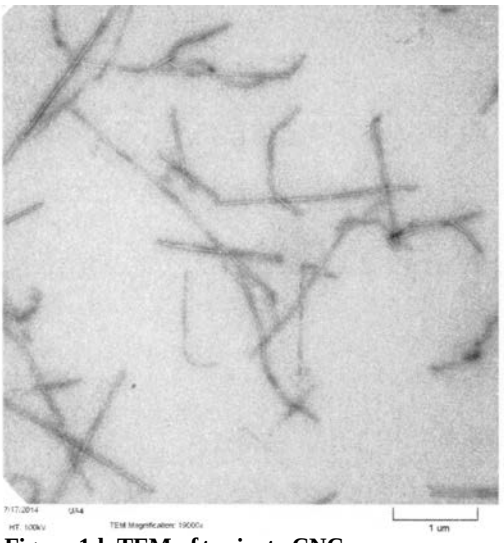

**Figure 1d. TEM of tunicate CNCs.**

Although based on Fig. 1, in general, CNCs can be described as rod-like nano particle with a range of aspect ratios. However, their form can be from rod-like to slender needles (shorter with tapered ends). Additionally, in the case of pulp-CNCs, it has been reported that different conditions during acid hydrolysis can influence the size (length and width), of the prepared pulp-CNCs.[5]

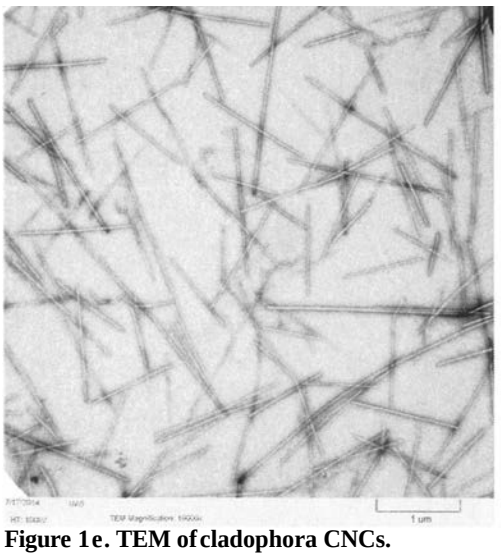

**Figure 1e. TEM of cladophora CNCs.**

**Raman analysis**

1064-nm Raman spectra of the CNCs were obtained, in $H_2O$ in $D_2O$, and in dried state. Analysis in the never dried state was carried out to understand the phenomenon of aggregation of CNCs. In general, the spectra were better resolved in the wet states. One particular Raman region of interest was the C-H stretch region because it is known from the Raman literature that the spectral characteristics of the C-H stretch modes depend upon the environment.[6] In Table 2, spectral features of the CNCs in the C-H stretch region are compared. Compared to the C-H stretch modes of Iß CNCs (tunicate, cotton, and pulp) which were at 2929 and 2896 $cm^{-1}$, for the Iα CNCs (cladophora and bacterial), the frequencies of these modes were significantly lower (2923, and 2891 $cm^{-1}$, respectively). Such differences were indicative of differences between the environments of the CH2 and CH groups (perhaps Iα vs. Iβ) and were analogous to what has been observed before between these two classes of celluloses (corresponding authors' unpublished results). Additionally, for pulp-CNCs, the intensity of the 2896 $cm^{-1}$ band was significantly higher compared to the other CNCs and the shoulders at 2871 and 2854 $cm^{-1}$ were not discernable.

**Table 2. Comparison of the C-H Raman frequencies ($cm^{-1}$) between different CNCs**

| Tunic. | Clado. | Bacter. | Cotton | Pulp |
|---|---|---|---|---|
| 2968, m[a] | 2968, m | 2968, m | 2968, m | 2968, m |
| 2943, sh | 2943, sh | 2943, sh | 2943, sh | 2943, sh |
| 2929, sh | 2923, sh | 2923, sh | 2929, sh | 2929, sh |
| 2907, sh | 2907, sh | 2907, sh | 2907, sh | — |
| 2896, vs | 2891, vs | 2891, vs | 2896, vs | 2896, vs |
| 2871, sh | 2871, sh | 2871, sh | 2871, sh | — |
| 2854, sh | 2854, sh | 2854, sh | 2854, sh | — |

[a]vs very strong, m medium, sh, shoulder; "—" means not detected.

In the dried state of the CNCs, the order of spectral resolution (high to low) was as follows - tunicate = cladophora > bacterial cellulose > cotton > pulp. An example of this behavior is shown in Fig. 1 where Raman spectra, in the region 850 - 1550 $cm^{-1}$, of tunicate- and pulp-CNCs are compared. Although similar spectral behavior was seen in the other spectral regions, for the purpose of clarity, spectra are compared only in 850 - 1550 $cm^{-1}$ interval. The higher resolution in the spectrum of tunicate CNCs seems to be due to its higher crystallinity (Table 3). This therefore implies that CNCs that, contained relatively lower order cellulose chains had poorer spectral resolution.

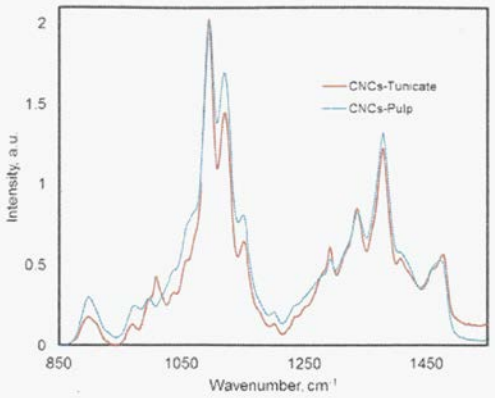


**Figure 1. Comparison of spectral resolution between the CNCs of tunicate and pulp in the Raman region 850-1550 $cm^{-1}$.**

Based on analysis of the $CH_2$ bending region in the Raman spectra of CNCs (1450 - 1480 $cm^{-1}$) which provides information on the various conformers of the $CH_2OH$ group in cellulose,[7] it was found that proportion of the *gt* and *tg* conformers varied in the CNCs. For example, compared to tunicate and cladophora CNCs, the concentration of the *gt* conformers in pulp and bacterial CNCs was much higher. This suggested existence of the conformational differences at the local level (monomer unit level). In Raman, the conformational differences were also found to be responsible for the intensity differences for some specific bands. Moreover, spectral differences between wet and dried states of the same CNCs were noted which could be interpreted in terms of conformation change that resulted upon drying.

Crystallinities of the CNCs and original celluloses are listed in Table 3. The crystallinities were based on the spectra that were obtained from the pellets of the freeze-dried materials. Upon comparison of the data in Table 3, it was noted that compared to the crystallinities of the original celluloses the crystallinities of bacterial- and pulp-CNCs were higher whereas the opposite was true in the case of the CNCs of tunicate, cladophora, and cotton. In the case of pulp-CNCs, the presence of hemicellulose was not an issue because its contribution had been corrected for during estimation of the 380-Raman crystallinity.[9] To further investigate this issue, crystallinities of the CNCs were also obtained in the never-dried state and it was concluded that drying did affect the values of Raman crystallinities. Therefore, it is possible that the manner in which the CNCs are dried and consolidated affects different CNCs differently. This, once again, points to the likelihood that differences between the organizations of cellulose chains within the CNCs dictate how the aggregation occurs. This topic needs to be further investigated.

**Table 3. 380-Raman[a] crystallinities of CNCs**

| CNCs, Sample ID | CrI, CNCs | CrI, original |
|---|---|---|
| Tunicate | 69.9 ± 2.1 | 86.5 |
| Cladophora | 72.1 ± 3.9 | 82.5 |
| Bacterial | 77.3 ± 0.7 | 65.6 |
| Cotton | 77.1 ± 1.0 | 82.2 |
| Pulp | 64.5 ± 0.2 | 53.2 |

[a]Raman crystallinity estimates were obtained using the methods of Agarwal et al.[8,9]

When the The X-ray based method (Segal-WAXS) was used to estimate crystallinity of the CNCs the trend observed above (Table 3) was confirmed. As an example, based on the XRD in Fig. 2, the Segal-WAXS CrIs of, tunicate, tunicate-CNCs, and pulp-CNCs were obtained to be 95.5, 88.4, and 82.8% respectively. This indicated that indeed the crystallinity of the tunicate-CNCs was lower compared to the tunicate cellulose.

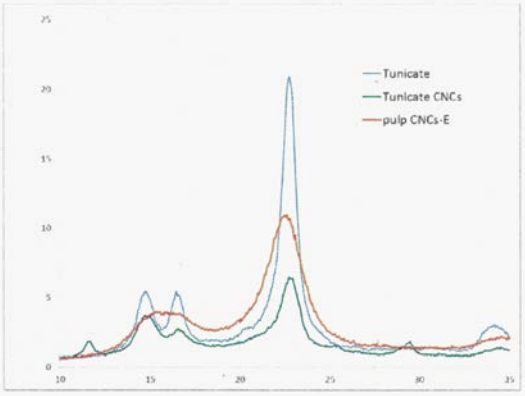


**Figure 2. X-ray diffractograms of tunicate cellulose, tunicate-CNCs and pulp-CNCs.**

Accessibility of the CNCs was measured by using the intensity increase at 1380 $cm^{-1}$ Raman band when the CNCs were samples in $D_2O$.[10] This Raman method was developed recently by the corresponding author and measures the % of water accessible $CH_2OH$ groups in cellulose.. Such an analysis when performed on the CNCs indicated that compared to tunicate CNCs pulp CNCs were 85% more water accessible. Although some of this difference can be explained by the difference in the crystallite sizes of the two types of CNCs. However, beyond size, it also indicated presence ,of more disordered cellulose in the case of the pulp-CNCs.

## CONCLUSIONS

CNCs obtained from a number of cellulose sources (tunicate, cladophora, bacteria, cotton, and wood-pulp) were analyzed and information on their supramolecular structures was generated using Raman spectroscopy. It was found that molecular level differences existed in between the CNCs and some of these were carried over from the original differences that existed in the source materials themselves. Additionally, crystallinity measurements on various materials showed that compared to original cellulose, CNCs crystallinity went up only in the case of bacterial- and pulp-CNCs. For the rest, the crystallinity declined upon CNCs production.

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