Sonication amplitude and processing time influence the cellulose nanocrystals morphology and dispersion

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ABSTRACT
The application of bio-based materials is becoming impellent owing to the increasing demand for alternatives to petroleum-based analogs. In this regard, cellulose nanocrystals (CNCs) with unique properties have received a significant interest, while their hydrophilic character poses a challenge to their commercial applications. Ultrasonication treatment is one of the most commonly used methods to improve CNCs’ dispersion in different solvents and polymer matrices. In this work, the effectiveness of ultrasonication treatment in the dispersion of CNCs in a water-soluble polymer (polyvinyl alcohol, PVA) was studied. An aqueous suspension of polyvinyl alcohol and CNCs was prepared using different ultrasonication times and amplitudes. The morphology, particle size and dispersion of CNCs were studied using X-ray diffraction, transmission electron microscopy, and dynamic light scattering. The results indicated that with increase in the sonication amplitude, there was a substantial decrease in nanoparticle length, while long sonication times gently affected the nanoparticle length. Furthermore, improved dispersion was observed in samples prepared using longer sonication time.

1. Introduction
During the last decade, numerous studies have been focused on the development and implementation of biopolymers owing to an increased concern over environmental sustainability and biocompatibility. Among different biopolymers, cellulose is one of the most abundant and naturally occurring polymers in the world which can be isolated from a wide range of renewable resources such as algae, bacteria, plants, and tunicates.

Cellulosic materials with different shapes, sizes and crystallinity can be produced through different treatments including acid hydrolysis, mechanical shearing and enzymatic hydrolysis [1–5]; cellulose...
nanocrystals (CNCs) are rod-like shaped structures containing only crystalline domains of cellulose. They can be extracted from cellulose through two steps; the first step includes the complete or partial removal of matrix materials such as hemicelluloses and lignin [6]. In the second step, which is a controlled acid hydrolysis, the amorphous regions of the cellulose polymer are removed which results in single and highly crystalline nanofibers. CNCs are of highly desirable due to their unique features, such as high aspect ratio of 10-70, high surface area of 150 m²/g, and high strength and modulus (10 and 150 GPa, respectively), depending on the method of isolation and the source of cellulose [7].

The presence of a large number of hydroxyl groups on the surface of the CNCs and intermolecular and intramolecular hydrogen bonding creates the challenge of utilizing CNCs as advanced materials for commercial applications. The abundant hydrogen bonds of cellulose draw the cellulose nanocrystals together and result in the high tendency to form bundles or aggregates [8]. So far, several modification techniques have been proposed for altering the hydrophilic character of CNCs and improving their dispersion in different solvents and polymer matrices. In addition to chemical surface modification treatments, the application of mechanical techniques has been reported to improve the dispersion of CNCs in different media [9].

Among different mechanical pre-processing techniques, ultrasonication has been widely employed as a practical step for improving the dispersion of CNCs in polymer matrices. In the ultrasonication process, the sonic waves are applied to a CNC suspension to break apart and de-agglomerate the CNC bundles. In the sonication process, ultrasound waves are applied to specimens with frequencies in the non-audible range (>20 kHz, 20,000 cycles per second) and as frequency increases the strength of the agitation increases.

In the ultrasonication process, the sound waves that propagate into the liquid generate alternating high-pressure (compression) and low-pressure (rarefaction) cycles, with rates depending on the frequency [10]. In the low-pressure cycle, small vacuum bubbles or voids are formed in sonicated liquid as a result of high-intensity ultrasonic waves. These bubbles reach a maximum volume at which they can no longer absorb energy and then violently breakdown during a high-pressure cycle (Figure 1).

The bubbles breakdown, which is called cavitation, creates dramatic effects in the sonicated liquid and give rise to pressures up to 1000 atm and temperatures of up to 5000 K [11]. Acoustic cavitation impact is strong enough to split up the fine particle agglomerates and disperse them more uniformly in liquids [12]. In the sonication process, sonication power, amplitude, time, and probe surface area describe the amount of energy delivered to the suspension, and inconsistent application of ultrasonic treatment is more likely to contribute towards variability in the results. Although sonication is a well-introduced technique to promote the dispersion of nanofillers in different solvents, there are very few reports explaining the effects of sonication inputs such as power and time on the dispersion of CNCs in an aqueous medium.

In this study, an aqueous suspension of polyvinyl alcohol (PVA) and CNCs was prepared using different ultrasonic treatments to investigate the dispersion of CNCs in a polymeric matrix as a function of different energy input and sonication time. Polyvinyl alcohol (PVA) is a biocompatible and biodegradable non-toxic synthetic polymer with application in diverse fields such as biomedical, food packaging and absorbents. To the best of our knowledge the ultrasonic treatments of CNCs in the presence of aqueous solutions of dilute PVA has not been described previously. Due to the large number of hydroxyl groups in PVA, strong interfacial hydrogen bonds can be formed between PVA and CNCs, making a completely miscible polymeric nanocomposite. However, the high tendency in forming aggregates in CNCs still required the application of different methods such as ultrasonic treatment to improve their dispersion quality even in a water soluble polymer.

2. Experimental

2.1. Materials

Unmodified freeze-dried cellulose nanocrystals extracted from pine wood fibers were supplied by Forest Product Society (Madison, WI, USA). Polyvinyl alcohol (PVA) with the molecular weight of 89,000-98,000 and the degree of hydrolysis of 99% was purchased from Sigma Aldrich (St. Louis, MO, USA).
2.2. CNC suspension and ultrasonication process

Aqueous PVA-CNCs suspension was prepared by dispersing equal amounts of 1\% wt. of PVA and CNCs powder in distilled water using a homogenizer (IKA, Ultra-turrax T25, Germany) at room temperature (23°C). An ultrasonic processor (Hielscher UIP1000hd, 20 kHz, Germany) with a 19 mm diameter probe was used to obtain a dispersion containing individual CNC nanoparticles. PVA-CNCs suspension (100 ml) was processed at a constant frequency of 20 kHz and two different levels of amplitude (60 and 90 \textmu m) and three time periods (4, 7, 10 min) in order to study the effect of sonication time and amplitude on CNCs dispersion in polymer matrix. The ultrasonication energy input was controlled by adjusting the amplitude of the sonicator probe. During the sonication process, samples were kept in an ice bath to ensure that the PVA-CNC suspension did not heat up and degrade the matrix. The ultrasound probe was submerged to a depth of 20 mm in the samples. The sample codifications and preparation conditions are shown in Table 1.

### Table 1. Codification and combinations of time and amplitude of ultrasonication force applied on PVA-CNC samples.

| Sample code | Time (min) | Amplitude (\textmu m) |
|-------------|------------|-----------------------|
| T4-A60      | 4          | 60                    |
| T4-A90      | 4          | 90                    |
| T7-A60      | 7          | 60                    |
| T7-A90      | 7          | 90                    |
| T10-A60     | 10         | 60                    |
| T10-A90     | 10         | 90                    |

2.3. Characterization of CNCs

2.3.1. Dynamic light scattering (DLS)

It is a non-invasive method which measures the translational diffusion coefficient of dispersed particles, undertaking Brownian motion. The mean particle size and particle size distribution for the dispersed cellulose nanocrystals were determined using a dynamic light scattering instrument (DLS-ZP/Particle Sizer Nicomp\textsuperscript{TM} 380 ZLS). The detection was made at a scattering angle of 90° and the viscosity value for water was used in all measurements. Each measurement was performed in triplicate, and mean values are reported.

2.3.2. Transmission electron microscopy (TEM)

The morphology and the crystalline nature of CNCs were further studied using high-resolution transmission electron microscopy (HRTEM) using JEOL JEM-100 CX II transmission electron. The suspensions were diluted 50x with distilled water and the nanofillers were stained with positively charged dye uranyl acetate prior to imaging. The sample for HRTEM was prepared by placing a drop of the aqueous suspension on carbon-coated copper grids under suitable conditions.

2.3.3. X-ray diffraction (XRD)

Crystallinity index (CrI) is a parameter commonly used to calculate the percentage of crystallinity in cellulosic materials. X-ray diffraction analysis is an analytical method which can quantify CrI using Segal’s method, as shown in the following equation [13]:

\[
CrI = \frac{I_{002} - I_{am}}{I_{002}}
\]

where \(I_{002}\) is the maximum intensity of the peak for the crystalline material at 2\(\theta\) = 22.5° and \(I_{am}\) is the intensity at 2\(\theta\) of 18.5°, which represents amorphous material.

In this study, X-ray diffraction analysis was used to evaluate the effect of sonication times and amplitudes on the crystal structure of CNCs in PVA-CNCs suspensions. XRD data were explored using a Rigaku Smartlab X-ray diffractometer equipped with Cu K\(\alpha\) radiation source (\(\lambda = 0.154 \text{nm}\) at 40 kV and 40 mA from 10-50° (2\(\theta\) angle range) and 1°/min of scanning speed.

### Table 2. DLS measurements of CNCs in different PVA-CNCs suspensions.

| Sample code | Length (nm) | Aspect ratio |
|-------------|-------------|--------------|
| Unmodified CNCs | 175.2 ± 5.4 | 27.5 ± 6.3 |
| T4-A60      | 169.0 ± 11.8| 25.9 ± 8.1  |
| T4-A90      | 151.5 ± 8.9 | 24.2 ± 9.4  |
| T7-A60      | 165.4 ± 11.8| 24.4 ± 7.3  |
| T7-A90      | 135.3 ± 11.1| 20.6 ± 8.8  |
| T10-A60     | 139.6 ± 6.5 | 21.7 ± 6.2  |
| T10-A90     | 116.6 ± 7.3 | 17.9 ± 8.0  |

3. Results and discussion

3.1. Dynamic light scattering (DLS)

In this work the effect of sonication treatment on the CNCs morphology and dispersion characteristics in a water-soluble polymer matrix were studied. The particle size distribution of CNCs as a function of ultrasonication treatment was evaluated using DLS and the mean values of hydrodynamic length of particles are summarized in Table 2. DLS analysis shows that the hydrodynamic length values of CNCs obtained ranges from 116.6 to 169.0 nm, depending on the time and the amplitude of ultrasonication process. When the amplitude of the sonication process increases, the particle size of the CNCs substantially decreases. Amplitude represents the distance that sonicator tip can longitudinally fluctuate and by increasing the amplitude, cavitation intensity within liquid is also increased.

The reduction in the particle size of CNCs was mainly as a result of fiber breakage due to particle agitation [14] which happened at higher ultrasonication amplitude known as harsh condition. Time of sonication was another factor which governed the
amount of acoustic energy delivered to the suspension and higher sonication times slightly degraded the structure of the CNCs and this in turn, reduced the length of the CNCs.

Beside the length of individual CNCs, the effect of time and the amplitude of ultrasonication process on aspect ratio of CNCs was also studied and the results are summarized in Table 2. The aspect ratio of CNCs displays a similar trend as CNCs’ length is more vulnerable than the diameter of individual fibers [15]. Cellulose nanocrystals subjected to higher ultrasonication amplitude for the same time had lower aspect ratio than their counterpart processed at lower amplitude. The relatively high standard deviation in aspect ratio of CNCs confirmed the polydispersity of CNCs.

### 3.2. Transmission electron microscopy (TEM)

The extraordinary tendency of cellulose nanoparticles to form aggregates which result in sediment as microparticles after ultrasonication processing was studied using HRTEM images. Figure 2 illustrates the TEM images of PVA-CNCs suspensions after ultrasonication treatments. As expected, more CNC bundles were observed in the sample which was treated for 4 min (T4-A60 and T4-A90). However, the presence of individual CNC particles in the PVA-CNCs suspension showed evidence of improved dispersion of CNCs in T7-A90, in fact, the longer sonication time in T10-A60 and T10-A90 delivered greater ultrasound energy input, and this resulted in a more uniform dispersion of CNC in the polymer matrix. The results are in agreement with another study on particle size analysis using DLS and image processing software confirmed decreasing nanocellulose length in suspensions which were subjected to ultrasound wave for 10 min [16, 17]. As it was reported elsewhere, the long and aggressive ultrasonic treatment could destroy nanofiber structure and consequently damaging its crystallinity and mechanical properties [18].
3.3. X-ray diffraction (XRD)

The application of ultrasonication treatment on crystallinity index of CNCs was studied using XRD and the representative XRD diffraction pattern of each formulation is shown in Figure 3. PVA is known to be a semi-crystalline polymer in nature and the diffractogram exhibited a characteristic peak at $2\theta = 19.5^\circ$ assigned to the (101) crystallographic plane for semi-crystalline PVA [19]. A well-defined CNCs diffraction peak at $22.5^\circ$ was observed for samples sonicated for 4 min, while peak intensity decreased as ultrasonication treatment time and amplitude increased. The XRD curve of T10-A60 and T10-A90 showed a broad and weak peak in the $22^\circ$–$25^\circ$ region indicating that crystalline structure of CNCs altered as a result of harsh ultrasonication treatment.

The crystallinity index (CI) calculated for the samples prepared using different preparation methods is summarized in Table 2. In the crystallinity determination, using the Segal method, the intensity $I_{\text{am}}$ at $18.5^\circ$ was overlapping with the tail of the PVA peak. Therefore, to remove the PVA component diffractograms were deconvoluted. It can be observed that by increasing the sonication time and amplitude the crystallinity index of CNC decreased. Ultrasound energy transferred to the CNC destroyed the crystalline structure of CNC and lowered the crystallinity index (Table 3).

4. Conclusion

This study evaluated the effect of ultrasonication amplitude and time on cellulose nanocrystals morphology and dispersion in a water-soluble polymer. The particle size, crystallinity, morphology, and dispersion of CNCs were studied using dynamic light scattering (DLS), X-ray diffraction (XRD), and transmission electron microscopy (TEM). The results confirmed that ultrasonication time and amplitude had considerable impact on cellulose nanocrystals morphology, crystallinity, and their dispersion throughout the PVA matrix, while the amplitude effect was very profound. As expected, the measured particle size of CNCs sonicated for longer time (10 min) and higher amplitude (90 $\mu$m) was significantly lower than the rest of the samples. Moreover, high amplitude (90 $\mu$m) reduced the length of CNCs by 17% in comparison with lower levels of amplitude (60 $\mu$m) for samples sonicated for equal time. By increasing the sonication time and amplitude the crystallinity index of CNC decreased by 12% as ultrasound energy destroyed the crystalline structure of CNC. The results of this study can be helpful in optimizing the ultrasonication treatment process for promoting uniform dispersion of CNCs and controlling their morphology.

Acknowledgement

The authors would like to thank to the USDA-ARS, Cotton Production and Processing Research Unit in Lubbock, TX for their financial supports.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 3. Crystallinity index of CNCs in different PVA-CNCs suspension.

| Sample code | CI          |
|-------------|-------------|
| Unprocessed CNCs | 68.6        |
| T4-A60     | 67.8 ± 1.5  |
| T4-A90     | 62.1 ± 1.7  |
| T7-A60     | 66.1 ± 1.9  |
| T7-A90     | 60.5 ± 1.1  |
| T10-A60    | 65.6 ± 2.1  |
| T10-A90    | 56.3 ± 1.3  |

CI: Crystallinity Index.
membranes, and conversion of biomass into bio-based industrial products. She has presented her research in several national and international conferences and meetings and has authored numerous research papers and technical reports, in various journals and books.

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