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1 **Impact of sonication on the rheological and colloidal properties of**  
2 **highly concentrated cellulose nanocrystal suspensions**

3

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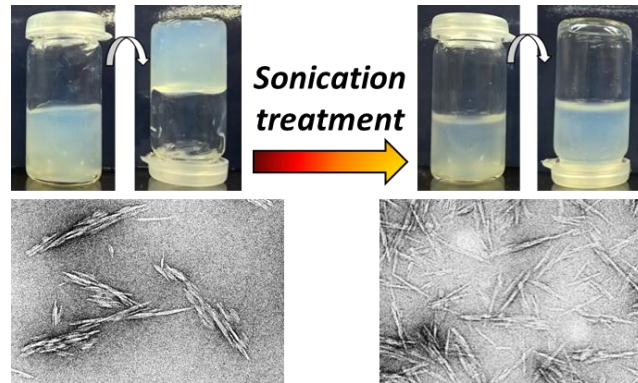
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## ABSTRACT

25 The effect of sonication on a highly concentrated commercial suspension of cellulose  
26 nanocrystals (CNCs) and the resulting rheological properties have been investigated.  
27 Rheology and structural analysis techniques (atomic force microscopy, small-angle X-ray  
28 scattering, transmission electron microscopy and dynamic light scattering) were used to  
29 characterize the CNC suspension before and after sonication as a function of concentration.  
30 The highly concentrated CNC suspension, which does not contain aggregates, as shown by  
31 AFM and TEM imaging, turns from a “gel” form into a “liquid” form after a sonication  
32 treatment. The self-organization properties of as-prepared and sonicated suspensions were  
33 compared by the determination of their phase diagrams and flow rheology was performed to  
34 understand the viscosity behavior as a function of concentration for both systems. Sonication  
35 induced a decrease of the inter-particle distance, a strong decrease of the viscosity and  
36 remarkable changes in the liquid crystalline behavior, while sonicated and non-sonicated  
37 suspensions were stable over time. These effects can be attributed to a decrease in the aspect  
38 ratio of the suspended particles, which varies from a high value before sonication due to the  
39 presence of elongated bundles to a lower value after sonication that promotes  
40 individualization.

41 **Keywords:** *Cellulose nanocrystals (CNCs), rheology, sonication, self-organization, aspect*  
42 *ratio*

43

## 44 **Introduction**

45 As biodegradable, renewable, non-toxic and high-performance nanomaterials, cellulose  
46 nanocrystals (CNCs) have evolved from a laboratory curiosity to a technical material, which  
47 has attracted industrial attention. These rod-like nanoparticles initially reported in the 1950's  
48 are commonly obtained by acid hydrolysis of cellulosic material at about 2-5% solid content  
49 (Rånby, 1951; Revol et al., 1992). CNCs are now produced in several countries at pilot or  
50 commercial scale by various companies since the 2010's (Reid et al., 2017). Their size, shape  
51 and surface properties both depend on the origin of the cellulose source and on the hydrolysis  
52 conditions (Beck-Candanedo et al., 2005; Bras et al., 2011; García et al., 2016). Owing to  
53 their specific physical properties and high aspect ratio, CNCs are used in the development of  
54 several applications in various fields (Tang et al., 2017) such as the biomedical domain (Jorfi  
55 and Foster, 2015; Lin and Dufresne, 2014; Sunasee et al., 2016), environmentally friendly  
56 nanocomposites (Mariano et al., 2014; Oksman et al., 2014; Siqueira et al., 2010) or the paper  
57 coating industry (Gicquel et al., 2017; Hoeng et al., 2017). In each case, the rheological  
58 properties and their variation with concentration play a major role. Recently, new processes  
59 have been proposed to favor industrialization and new grade delivery. Highly concentrated  
60 suspensions (up to 11 wt%) are now commercially available (Reid et al., 2017). This study  
61 focuses on this grade.

62 As other colloidal rods like DNA fragments (Hamley, 2010), viruses (Watson, 1954), metal  
63 oxides (Zhang et al., 2005) or chitin nanocrystals (Revol et al., 1992), CNC suspensions  
64 exhibit very interesting self-organization properties: above a critical concentration, the  
65 suspension phase-separates into an upper isotropic phase and a lower anisotropic chiral  
66 nematic phase (Revol et al., 1992, 1994). The formation of this phase can be explained by  
67 Onsager's theory (Onsager, 1949), which predicts the critical transition density for very high  
68 aspect ratio hard rods. This theory was further refined to take into account smaller aspect  
69 ratios, polydispersity and most importantly electrostatic interactions (Araki and Kuga, 2001;  
70 Dong and Gray, 1997; Lee, 1987). The dimensions, aspect ratio, surface charge density and  
71 surface chemistry greatly influence the phase separation behavior and the characteristics of  
72 the cholesteric liquid-crystalline phase (Abitbol et al., 2018; Azzam et al., 2016; Lagerwall et  
73 al., 2014; Revol et al., 1994; Shafiei-Sabet et al., 2014).

74 The rheological behavior of aqueous CNC suspensions stands as a major concern of high  
75 application-relevance and different studies over the past 10 years were dedicated to its

76 comprehensive understanding (Bercea and Navard, 2000; Liu et al., 2011a, 2011b; Lu et al.,  
77 2014; Shafiei-Sabet et al., 2012, 2014; Ureña-Benavides et al., 2011; Xu et al., 2017a, 2017b).  
78 One of the important reported results is the effect of concentration and the related position in  
79 the phase diagram. While isotropic suspensions behave like classical polymer solutions, the  
80 viscosity profile as a function of shear rate observed for liquid crystalline and biphasic CNC  
81 suspensions decomposes into three distinct regions (Bercea and Navard, 2000; Shafiei-Sabet  
82 et al., 2012). Namely, at low shear rate, a first shear-thinning region due to the alignment of  
83 chiral nematic domains is observed. At intermediate shear rates, a plateau with constant  
84 viscosity is observed. It is corresponded to the alignment of all chiral nematic domains in the  
85 flow direction. Finally, at high shear rate, chiral domains are disrupted and all CNCs align in  
86 the flow, leading to a second shear-thinning region.

87 Nonetheless, most studies have focused on low concentration suspensions or at least have  
88 used such suspensions as starting material. Furthermore, limited information exists on the  
89 relationship between macroscopic rheological properties and the microscopic structure of  
90 CNCs suspensions whatever the concentration is. A few works involve rheology coupled with  
91 scattering techniques such as small-angle neutron, small-angle X-ray and light scattering  
92 (SANS, SAXS, and SALS, respectively) to characterize the orientation and morphology of  
93 CNCs under flow (Ebeling et al., 1999; Orts et al., 1998; Schütz et al., 2015; Xu et al.,  
94 2017a). Orts et al. (Orts et al., 1998) showed that CNC rods are oriented parallel to shear  
95 flows with increasing shear rate.

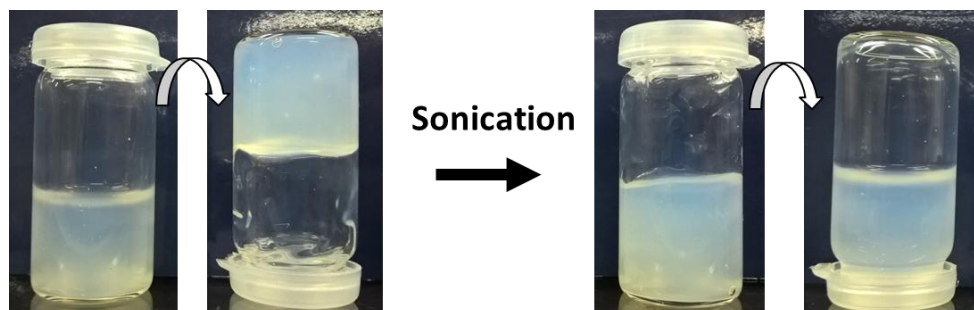
96 Ultrasound treatment (also called sonication) is ordinarily used at the end of the process of  
97 creation of CNCs and also to disperse CNC aggregates in dilute suspension (Beuguel et al.,  
98 2018; Csiszar et al., 2016; Dong et al., 1996), which generally causes a decrease in viscosity  
99 in dilute conditions (Beuguel et al., 2018; Dong et al., 1998; Marchessault et al., 1961;  
100 Shafiei-Sabet et al., 2012). This viscosity variation is important and, to our knowledge, the  
101 effect of sonication on the rheological behavior and microstructure (like inter-particle  
102 distance) of highly concentrated CNC suspension has not been studied in details.

103 Beck et al. (Beck et al., 2010) evaluated the impact of ultrasound energy on CNCs in dilute  
104 conditions (~3 wt%) to prepare colored solid films and control their optical properties. The  
105 authors found that the chiral nematic pitch in the CNC films increased with increasing  
106 sonication energy input resulting in a change in the film color. Shafiei-Sabet et al. (Shafiei-  
107 Sabet et al., 2012) investigated the effects of concentration, temperature and sonication on the

108 microstructure and rheological behavior of aqueous CNCs suspension. They prepared  
109 suspensions from freeze-dried CNCs and reached a maximum concentration of  $\sim 7$  wt%. They  
110 found that the minimum amount of sonication necessary to disperse aggregates was  $1000 \text{ J}\cdot\text{g}^{-1}$   
111 whatever the concentration. Nonetheless, they did not observe any cholesteric phase  
112 formation before sonication. Beuguel et al. (Beuguel et al., 2018) investigated the impact of  
113 ultrasonication on dilute CNCs suspension ( $< 5$ wt.%) after spray- and freeze-drying process.  
114 They explained that sonication break aggregates and release ions/water but the energy  
115 generated by sonication was not sufficient to desulfate CNCs. Csiszar et al. (Csiszar et al.,  
116 2016) evaluated the impact of sonication time at the end of the preparation process on the  
117 CNCs size and on the transparency of CNCs films. Honorato-Rios et al. (Honorato-Rios et al.,  
118 2018) recently examined the impact of salt on liquid crystal behavior after sonication of  
119 CNCs suspensions over a broad range of concentration.

120 In the present study, never dried highly concentrated CNC suspensions have been submitted  
121 to a sonication treatment which induced a clear change in the rheological behavior (**Fig. 1**).  
122 The non-sonicated suspension existed in a stable gel-like state, while after sonication, the  
123 suspension was liquid and able to flow when the vial was turned upside down. Besides,  
124 cholesteric domains could form before the sonication treatment (**Support information S1**). In  
125 order to highlight the origin of this phenomenon, we have studied the impact of sonication on  
126 the morphology, self-organization properties and rheological behavior of CNCs using  
127 suspensions with concentrations spanning over a wide range (from 1 to 12 wt%).  
128 This article is part of the Ph.D. thesis “*Development of stimuli-responsive cellulose*  
129 *nanocrystals hydrogels for smart applications*” by Erwan GICQUEL (Gicquel, 2017).

130  
131



132  
133 **Fig. 1** Effect of sonication ( $5000 \text{ J/g}_{\text{CNC}}$ ) on the flow properties of a 12 wt% CNC suspension.

134

## 135 **1. Materials and Methods**

### 136 **1.1. Materials**

137 The commercial cellulose nanocrystals (CNCs) used in this study were purchased from  
138 UMaine Development Center (Forest Products Laboratory, USA) and used as received. These  
139 CNCs have been produced from wood pulp using sulfuric acid hydrolysis. The colloidal  
140 suspension was never freeze-dried and the dry matter content determined with a moisture  
141 analyzer (Ohaus® MB-35, Sigma-Aldrich, USA) was ~12 wt%. The sulfur content of the  
142 suspension is 1 wt.% as determined in recent article (Honorato-Rios et al., 2018; Xu et al.,  
143 2017b).

### 144 **1.2. CNC suspension characterization**

145 **Atomic force microscopy (AFM):** CNCs were imaged using AFM (Dimension icon®,  
146 Bruker, USA). All suspensions were previously diluted to  $10^{-4}$  wt% and a drop of 0.2 mL was  
147 deposited onto a freshly cleaved mica plate. Samples were dried overnight at room conditions.  
148 The samples were characterized in tapping mode using a silica coated cantilever (OTESPA®  
149 300 kHz - 42 N/m, Bruker, USA). Scans of  $10 \times 10 \mu\text{m}^2$  and  $3.3 \times 3.3 \mu\text{m}^2$  were performed to  
150 analyze the dimensions of CNCs. At least 4 images per sample were obtained. In order to  
151 extract the length and width dimensions, measurements of about 200 particles were performed  
152 by using the ImageJ software to obtain a representative average size.

153 **Transmission electron microscopy (TEM):** Drops of about 0.001 %wt CNC suspensions  
154 were deposited onto glow-discharged carbon-coated TEM grids. After 2 min, the liquid in  
155 excess was absorbed with filter paper, and prior to drying, a drop of Uranyless negative stain  
156 (Delta Microscopies, France) was deposited. After 2 min, the solution in excess was blotted  
157 and the grid was air-dried. The specimens were observed using a Philips CM200 microscope  
158 (FEI, USA) operating at 200 kV. The images were recorded with a TVIPS TemCam F216  
159 digital camera. In order to measure the length of the particles. About 150 particles were  
160 measured from the images using ImageJ and calculate a representative average size.

161 **Dynamic light scattering (DLS):** Dynamic light scattering measurements were performed on  
162 CNC suspensions using a Vasco® I DLS apparatus (Cordouan Technologies). Samples were  
163 diluted in deionized water to  $10^{-2}$  wt% and the conductivity was adjusted to  $500 \mu\text{S} \cdot \text{cm}^{-1}$  by  
164 addition of a NaCl solution. The hydrodynamic diameter ( $z^*$ ) and polydispersity index (PDI)

165 were calculated using cumulant analysis. For each sample, the final values represent an  
166 average of at least 3 acquisitions with 10 measurements.

### 167 **1.3. Small-angle X-ray scattering (SAXS)**

168 SAXS measurements were carried out at the ID02 TRUSAXS beamline of the European  
169 Synchrotron Radiation Facility (ESRF, Grenoble, France). A monochromatic X-ray beam at a  
170 wavelength of 0.995 Å was used. Scattered intensities were recorded on a two-dimensional  
171 CCD detector at sample-detector distances of 2 and 10 m in order to cover a  $q$  domain ranging  
172 from  $10^{-3}$  to  $10^{-1}$  Å<sup>-1</sup>. Samples were placed in flow-through cells (diameter 1.7 mm), and were  
173 left at rest for 5 min before acquisition. The background scattering of pure water and the  
174 capillary were subtracted prior to radial integration.

175 **Particle size:** The scattering intensity distribution  $I(q)$  as a function of the scattering vector  $Q$   
176 was obtained by radially integrating the two-dimensional (2D) scattering patterns. The  
177 particle length, width and thickness ( $a$ ,  $b$  and  $c$ , respectively) were extracted from the fitting  
178 of the scattering profile with the form factor of a parallelepiped using the SasView® 4.0.1  
179 modeling software.

180 **Interparticle distance:** A Kratky plot (corresponding to  $q^2 \cdot I(q)$  vs  $q$ ) analysis was  
181 performed to measure the average interparticular distance,  $d$ . In this representation:  $d =$   
182  $2\pi/q_{peak}$ , where  $q_{peak}$  is the position of the scattered intensity maximum.

### 183 **1.4. CNC dispersion and dilution**

184 **Non-sonicated suspensions:** To obtain a homogeneous and well-dispersed suspension, the  
185 desired weight concentration of the CNC suspension was diluted with deionized water (pH =  
186 6) with fixed ionic strength (18 mM or 1300 μs·cm<sup>-1</sup> obtained with addition of NaCl). Then,  
187 this suspension was magnetically stirred for at least 2 h.

188 **Sonicated suspensions:** The dilution was identical to that of non-sonicated dispersions. These  
189 suspensions were exposed to a dispersive energy of 5 kJ per g of dry CNCs using a 250 W  
190 sonication probe (Sonifier S-250A, Branson, USA) at 50% of maximum energy. To prevent  
191 thermal degradation of the CNCs during sonication, the temperature of the sample was control  
192 to keep room temperature without excess heating thanks to a container with ice.



193 **Mechanically-homogenized dispersions:** The dilution was identical to that of non-sonicated  
194 dispersions. The suspensions were then mechanically mixed with an Ultra-turrax (IKA T25  
195 digital) at a speed of about 5000 rpm.

### 196 **1.5. CNC suspension concentration**

197 **Sonicated suspensions:** The starting solution at 12 wt% was sonicated at 5 kJ per g of CNCs  
198 using a 250 W sonication probe (Sonifier S-250A, Branson, USA). Then, the suspension was  
199 concentrated with an EMD Millipore Amicon® bio-separation stirred cell (Merck KGaA,  
200 Darmstadt, Germany). The highest concentration that was reached was 17.6 wt%.

### 201 **1.6. CNC phase diagram**

202 **Non-sonicated suspensions:** Samples from 1 to 12 wt% (with 1 wt% steps) were poured into  
203 5 mL sealed glass vials and allowed to rest for at least 10 days at room temperature. These  
204 vials were observed between crossed-polars and pictures were recorded using a digital  
205 camera. The isotropic/anisotropic phase ratio was obtained from the height of each phase  
206 measured on these images.

207 **Sonicated suspensions:** The protocol was identical to that used for non-sonicated suspensions  
208 to prepare samples from 1 to 17.6 wt% (with 1 wt% steps).

### 209 **1.7. Rheological experiments on CNC suspensions**

210 The rheology behavior of the aqueous CNC suspensions under shear was studied using two  
211 different rotational rheometers: the Physica MCR 301 (Anton-Paar, Austria) and DHR3 (TA  
212 Instruments, New Castle, DE) instruments. The tests were carried out at a temperature of 20  
213 °C. Shearing tests were performed with a cone-plate configuration (diameter of 50 mm and  
214 angle of 1° for MCR 301 and diameter of 65 mm and angle of 2° for DHR3). The atmosphere  
215 around the sample was saturated with water to avoid evaporation during the measurement. To  
216 control the initial state of each sample, a constant shear rate was applied. The transient  
217 response under shear was recorded until steady conditions were obtained. Steady shear  
218 viscosity versus shear rate curves were generated for each sample from a shear rate of 0.01 to  
219 1000 s<sup>-1</sup>. The time required to reach steady conditions was decreased with increasing shear  
220 rate. Oscillatory measurements were also performed to collect the relaxation time and the  
221 elastic behavior of each sample. The percent strain used was inside the linear viscoelastic  
222 region (LVE) as determined by performing strain sweep experiments at a frequency of 1 Hz.  
223 Then the percent strain (obtained in the LVE) was fixed and oscillation sweep experiments

224 were performed. The frequency used for oscillatory measurements was also inside the LVE..  
225 These two values were fixed to obtain the storage modulus ( $G'$ , Pa) and the loss modulus  
226 ( $G''$ , Pa) before and after a strong shear deformation (up to  $100 \text{ s}^{-1}$ ).

## 227 **2. Results & Discussions**

### 228 **2.1. CNC morphology**

229 AFM and TEM images of CNC particles before and after sonication are shown in **Fig. 2**.  
230 These data reveal changes in morphology, size and size distribution upon the sonication  
231 treatment. Before sonication, the particles clearly exhibit a composite aspect and are formed  
232 of the lateral association of elementary rod-like crystallites (**Fig. 2b** and **c**). These particles are  
233 not aggregated but images reveal elongated objects composed of laterally associated  
234 elementary crystallites. In a particle, the constituting crystallites are all parallel and their  
235 number is estimated to range between 4 and 20 (determined from TEM images). The average  
236 length was  $217 \pm 42 \text{ nm}$ , measured from AFM and TEM images. The average height,  
237 determined from the AFM images, was  $10 \pm 5 \text{ nm}$  (**Fig. 2a**), a value which is rather close to  
238 the lateral size of the constituting crystallites and suggests that the particles have a lamellar  
239 shape.

240 After sonication, the particles appear to be smaller both in length and width, and the number  
241 of constituting crystallites has decreased to a few units (about 1 to 4) (**Fig. 2e** and **2f**). The  
242 average length was  $150 \pm 30 \text{ nm}$  (AFM & TEM images) and the CNCs' height was still  
243  $10 \pm 5 \text{ nm}$  (**Fig. 2d**). The values after sonication of the CNCs are in line with the literature  
244 data on CNCs from wood pulp (Habibi et al., 2010; Lagerwall et al., 2014; Shafiei-Sabet et  
245 al., 2012). Such a composite morphology of CNCs from different botanical sources has  
246 already been described by other authors (Chauve et al., 2014; Elazzouzi-Hafraoui et al.,  
247 2008). It is considered to originate from the strong lateral association of cellulose microfibrils  
248 after drying of the raw material. The strong acid hydrolysis and subsequent sonication  
249 treatments during the process of fabrication were not sufficient to break this association.

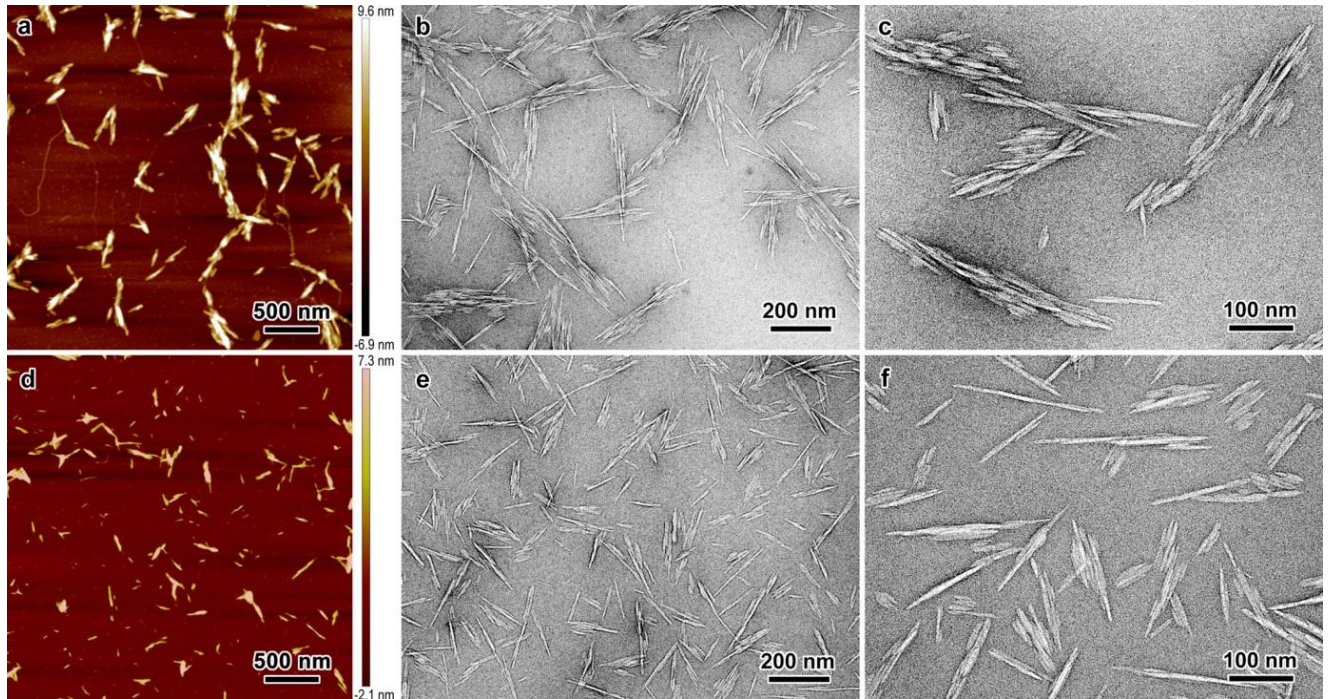
250 The decrease in dimensions was confirmed by DLS analysis, as the hydrodynamic diameter  
251 decreased from  $119 \pm 3$  to  $80 \pm 1 \text{ nm}$  after sonication (**Table 1**).

252 **Table 1** Hydrodynamic size (DLS) and size distribution (AFM & TEM: length, AFM: width) of  
253 sonicated and non-sonicated CNC suspensions. The standard deviations are indicated between  
254 parentheses.

	Hydrodynamic properties		Size	
	$Z^*$ (nm)	PDI	Average length (nm)	Height (nm)
Non-sonicated CNCs	119 (3)	0.20	217 (42)	10 (5)
Sonicated CNCs	80 (1)	0.17	150 (30)	10 (5)

255

256



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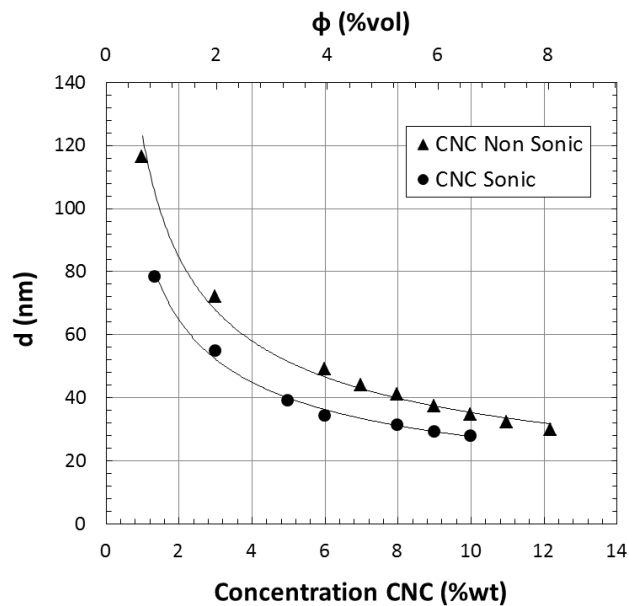
258 **Fig. 2** CNC morphology: a, b, c before sonication; d, e, f after sonication. a and d are AFM images  
 259 while b, c, e and f are TEM images of negatively stained preparations

## 260 2.2. Interparticle distance in static conditions

261 In order to investigate the effect of sonication on the structural features of the suspensions,  
 262 analyses at different length scales were performed by SAXS. **Fig. 3** shows the evolution of  
 263 the average distance between CNCs with the concentration of the suspension. With or without  
 264 sonication, the general behavior is the same and follows a power law decrease. With  
 265 increasing concentration, the interparticle distance quickly decreases and reaches a minimum  
 266 close to 30 nm in the concentration range probed. Both power law fits yield an exponent close  
 267 to  $-1/2$ , which is characteristic of the signal of long cylinders in hexagonal arrangement  
 268 (Baravian et al., 2010). The elongated shape of the CNCs is thus conserved through the  
 269 sonication treatment. However, during sonication the spatial distribution of particles is  
 270 modified and at a given cellulose volume fraction, the interparticle distance is lower after  
 271 sonication. This effect can be attributed to a sonication-induced scission of the objects that  
 272 would decrease their aspect ratio. This hypothesis is confirmed by the fact that curve

273 corresponding to non-sonicated CNCs can be shifted to superimpose with that of the  
 274 sonicated CNCs by considering a two-fold decrease in the aspect ratio.

275 The  $a$ ,  $b$  and  $c$  characteristic dimensions of CNCs were obtained by plotting the intensity  
 276 distribution  $I(q)$  as a function of scattering vector  $q$  and fitting the curves with different form  
 277 factor models. A parallelepiped model gives the best result, in agreement with already  
 278 reported SAXS and SANS data (Cherhal et al., 2015; Mao et al., 2017). Dimensions  $a$ ,  $b$  and  $c$   
 279 extracted from the fitting procedure are  $114 \times 60 \times 4 \text{ nm}^3$  before sonication and  
 280  $121 \times 20 \times 5 \text{ nm}^3$  after sonication. However, as already reported (Cherhal et al., 2015), only a  
 281 very poor accuracy in the length determination can be obtained due to the limited  $Q$ -range that  
 282 was probed. More accurate values could be obtained from investigations at much lower  $Q$ -  
 283 values. A high polydispersity appears in the calculation of the  $b$  dimension for non-sonicated  
 284 CNCs ( $\pm 10 \text{ nm}$ ), which is in line with AFM and TEM images showing objects with large  
 285 lateral size variations. To stay within confidence limits, it can be concluded that SAXS data  
 286 show that the sonication treatment induces a decrease in the width of the particles while their  
 287 height, of the order of the size of elementary crystallites, is unaffected.



288  
 289 **Fig. 3** Average CNC center-to-center interparticle distance  $d$  as a function of concentration (in wt%,  
 290 bottom scale) or volume fraction ( $\phi$  in vol%, top scale) in suspensions at different concentrations,  
 291 deduced from the Kratky plot ( $q^2 \cdot I(q) = f[q]$ ). CNC were exposed (●) or not exposed (▲) to  
 292 sonication. Interpolation curves correspond to a power law model. The power law equation is  $y =$   
 293  $93x^{-0.53}$  and  $y = 123x^{-0.54}$  for sonicated and non-sonicated CNC suspensions, respectively.

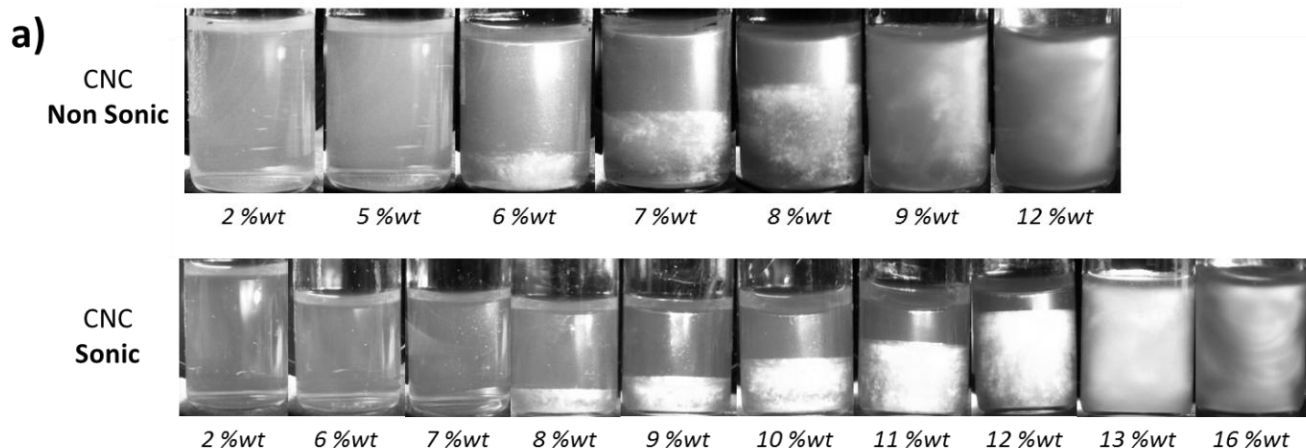
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### 295 2.3. Phase behavior and microstructure after sonication at high concentration

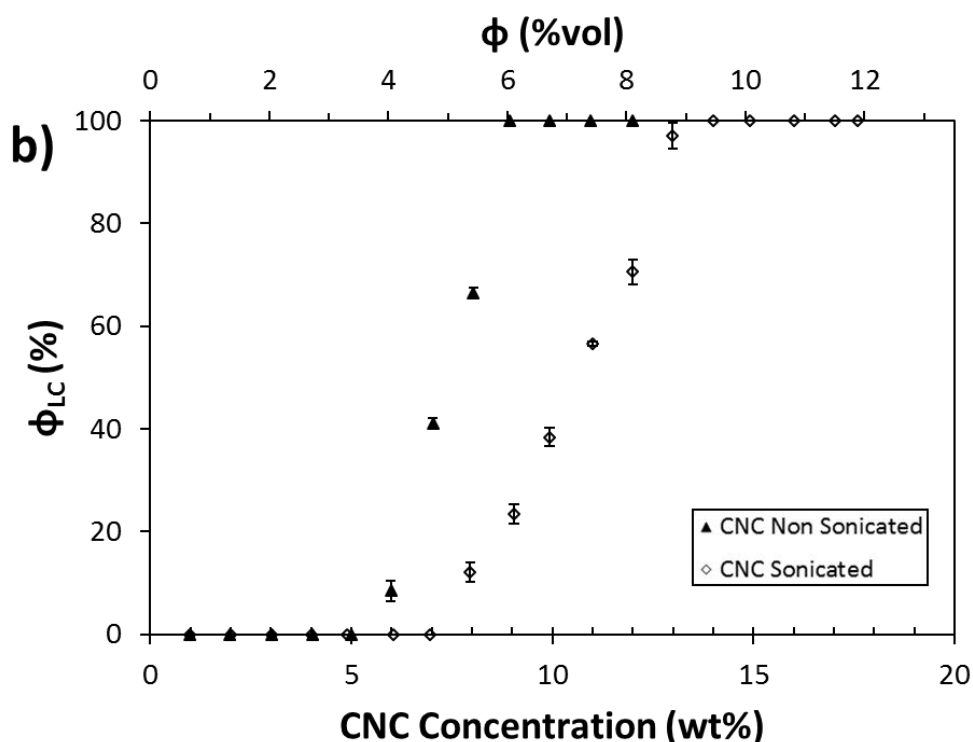
296 **Fig. 4** presents the phase diagrams for sonicated and non-sonicated CNC suspensions. In the  
297 first case, a sonicated suspension at a concentration of about 12 wt% has been used for sample  
298 preparation, while in the second case the as-delivered 12 wt% non-sonicated batch has been  
299 used. The first remarkable feature is that both types of suspensions exhibit a clear phase  
300 separation behavior: above a first critical concentration,  $C_i$ , biphasic samples are obtained  
301 with an upper isotropic phase and a lower birefringent anisotropic one and a fully anisotropic  
302 phase is observed above a second critical concentration,  $C_a$  (**Fig. 4**). However, the time  
303 required to reach equilibrium is strongly ultrasound treatment dependent. Depending on the  
304 concentration, it took 5 to 7 days for the non-sonicated suspensions to equilibrate, while for  
305 sonicated samples complete phase separation was reached within 20h only. The sonication  
306 treatment therefore strongly accelerates the phase separation kinetics.

307 The critical concentrations were measured in each case as the linear extrapolation in the  
308 biphasic gap at 0 and 100 vol % for the critical concentration,  $C_i$  and  $C_a$ , respectively.

309 The results show that sonication also has a huge influence on the critical concentration values  
310 with  $C_i \sim 5.8$  and  $7.5$  wt% and  $C_a \sim 9$  and  $13.3$  wt% for the non-sonicated and sonicated  
311 suspensions, respectively. Without sonication, phase separation occurs at lower concentration  
312 and the biphasic gap is thinner than with sonication. The biphasic gap in the case of the non-  
313 sonicated samples spans over 3.2 wt% (or  $C_a/C_i = 1.5$ ), which is an unusually small value  
314 compared to what is reported in the literature (Dong and Gray, 1997; Dong et al., 1996).  
315 Interestingly, the critical concentrations values reported by Xu et al., in the case of CNCs of  
316 the same origin but with samples prepared from the sonication-assisted redispersion in water  
317 of a freeze-dried powder, are  $C_i = 3.5$  and  $C_a = 10$  wt%, respectively. *i.e.* with a lower  $C_i$  value  
318 and a higher  $C_a$  value than in our non-sonicated case and a much lower  $C_i$  and lower  $C_a$  than  
319 for our sonicated system (Xu et al., 2017b, 2018). This comparison suggests that freeze-  
320 drying step could also influence the self-organization properties of this type of CNCs.



321



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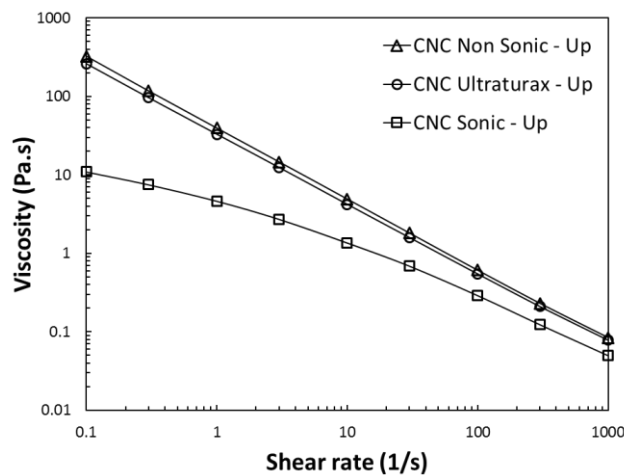
323 **Fig. 4** Phase behavior of aqueous CNC suspensions after equilibration. a) photographs of vials  
 324 between crossed polars showing the phase separation of CNC suspensions from 1 to 12 wt% (non-  
 325 sonicated CNCs) and from 1 to 16 wt% (sonicated CNCs). The lower liquid crystalline phase  
 326 (anisotropic) is white while the upper isotropic phase appears dark. b) Volume fraction of liquid  
 327 crystalline phase ( $\phi_{LC}$  in vol%) as a function of the solid weight in the CNC suspensions. ▲ and ◇  
 328 correspond to non-sonicated and sonicated CNC suspensions, respectively. The error bars represent  
 329 the standard deviation of the fitted Gaussian peaks.

#### 330 2.4. Influence of sonication on the rheological behavior

331 To complete the static and microstructure studies, the rheological behavior of a commercial  
 332 suspension at 12 wt% was firstly investigated.

333 **Effect of mechanical treatment on highly concentrated CNC suspension rheology**

334 **Figure 5** compares the effect of sonication and mechanical homogenization on the rheological  
335 behavior of CNC suspensions at 12 wt% after several months of storage at optimum condition  
336 in fridge. The non-sonicated initial CNC suspension exhibits a shear-thinning behavior. When  
337 the shear rate increases from 0.1 to 1000 s<sup>-1</sup>, the viscosity drastically decreases from 300 to  
338 0.08 Pa.s, and follows a  $\nu = 49.65 \dot{\gamma}^{0.06}$  power law . As shown in **Fig. 1**, the suspension in a  
339 gel-like form did not flow when the vial was turned upside down.



340  
341 **Fig. 5** Flow curves of 12 wt% CNCs suspensions as-received and treated by sonication or by  
342 mechanical homogenization.

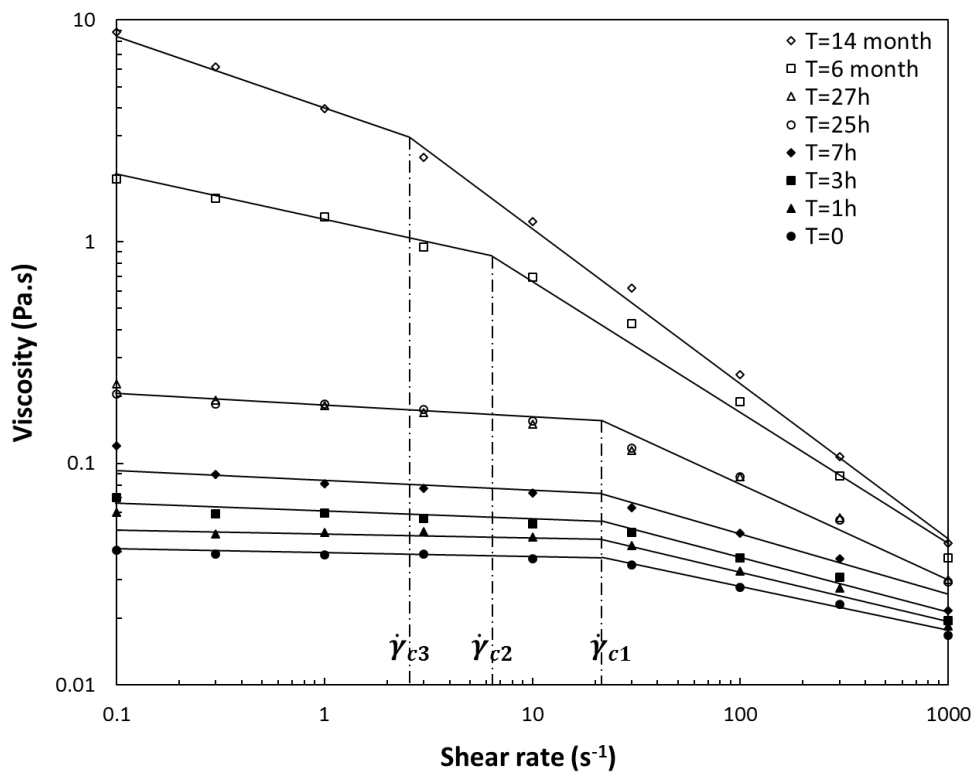
343 After a strong mechanical homogenization, the CNC suspension does not exhibit any change  
344 in behavior compared to the non-sonicated suspension. However, sonication drastically  
345 affects the viscosity, and a more than 10-fold-decrease in viscosity was observed in the low  
346 shear-rate region. Accordingly, as shown in **Figure 1**, the sonicated suspension was liquid  
347 and flowed when the vial was turned upside down.

348 **Influence of aging time after sonication**

349 **Fig. 6** presents the evolution with time of the rheological behavior of a 12 wt% CNC  
350 commercial suspension after ultra-sonication treatment. Before sonication, the rheological  
351 behavior for the same suspension does not show any time evolution (data not shown). In this  
352 figure, two behaviors are identified and separated by a relaxation time-dependent critical  
353 shear rate,  $\dot{\gamma}_c$ . Below this value, a viscosity plateau is obtained. Then, at higher shear rate, a  
354 shear-thinning behavior is observed.  $\dot{\gamma}_c$  decreases when the relaxation time after sonication  
355 increases. In details, just after sonication, the maximum viscosity at low shear rate is close to  
356 0.04 Pa.s and decreases to 0.016 Pa.s at 1000 s<sup>-1</sup>. During the following hour, the critical shear

357 rate  $\dot{\gamma}_c$  does not change ( $20 \text{ s}^{-1}$ ) but the viscosity slightly increases. The characteristic value  $\dot{\gamma}_c$ ,  
 358 and the general viscosity change after one day of relaxation. As aforementioned, the biphasic  
 359 domain reaches equilibrium within one day. The viscosity increases because of a  
 360 reorganization of the CNCs from an isotropic phase to an isotropic/anisotropic biphasic  
 361 structure. After more than a year of relaxation the viscosity at low shear rate increases to 9  
 362 Pa·s and  $\dot{\gamma}_c$  decreases to  $2.5 \text{ s}^{-1}$ .

363 It has to be noted than despite the described evolution with time, the suspension never comes  
 364 back to the rheological behavior of the non-sonicated suspension (plotted in **Fig. 5**). Recent  
 365 work of Beuguel et al. (Beuguel et al., 2018) explain that the use of ultrasonication on CNCs  
 366 suspension (from spray- and freeze-dried suspensions) will break aggregates and decrease the  
 367 viscosity. However, according to these authors, no desulfation takes place since this  
 368 phenomenon would require more energy than generated by sonication. The temporal  
 369 evolution of the rheological behavior after sonication could be explained by a reorganization  
 370 of cholesteric domains and/or a possible densification of the CNCs ions double layers (Beck  
 371 et al., 2010).

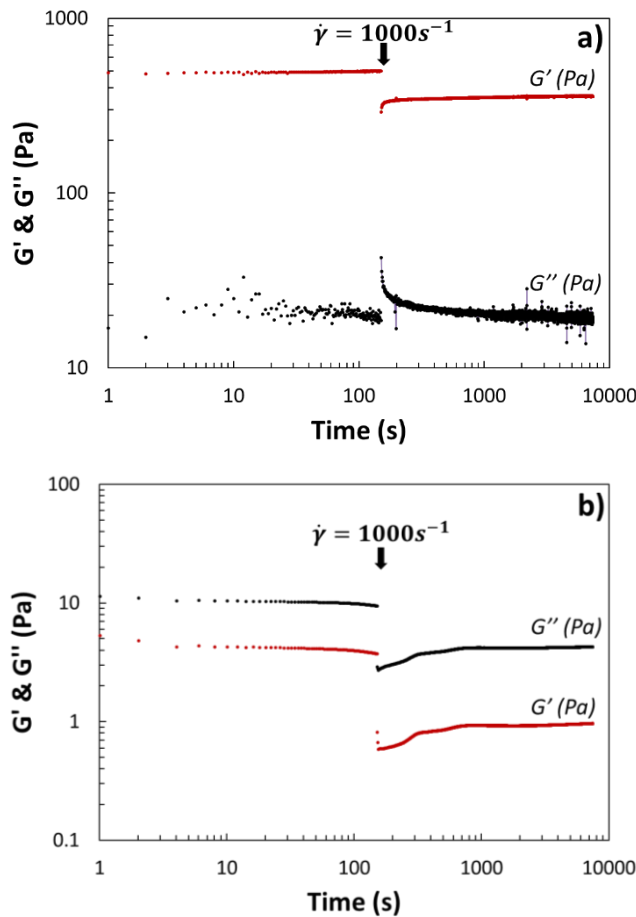


372 **Fig. 6** Flow curve evolution with aging time of sonicated 12 wt% CNCs suspensions. Critical shear  
 373 rate ( $\dot{\gamma}_c$ ) are indicated in dotted lines, respectively.  $\dot{\gamma}_{c1}$ ,  $\dot{\gamma}_{c2}$  and  $\dot{\gamma}_{c3}$  are equal to 20, 6 and  $2.5 \text{ s}^{-1}$ ,  
 374 respectively.  
 375



376 In order to understand the viscoelastic properties and the restructuration after shear rate of  
 377 both non-sonicated and sonicated 12 wt% suspensions, oscillatory rheology experiments were  
 378 performed. A constant strain within the linear viscoelastic (LVE) region was applied in order  
 379 to keep the structural organization unchanged and probe the non-flowing samples. A strong  
 380 deformation ( $\dot{\gamma} = 1000 \text{ s}^{-1}$ ) largely out of the LVE was then applied to break the system and  
 381 organize CNCs. Then, again, a constant strain within the LVE was applied.  $G'$  (elastic  
 382 modulus in Pa) and  $G''$  (viscous modulus in Pa) values quantify respectively the elastic and  
 383 viscous behavior of the CNC suspension.

384



385

386

387 **Fig. 7** Evolution of  $G'$  (Pa) and  $G''$  (Pa) for non-sonicated (a) and sonicated (aging time 24h) (b) 12  
 388 wt% CNC suspensions

389 With these values, the loss factor  $\tan \delta$  can be calculated according to **Equation 1**:

$$(1) \quad \tan \delta = G''/G'$$

390 In this equation, if  $\tan \delta$  is lower than 1, the elastic modulus ( $G'$ ) dominates and the  
 391 suspension behaves like a solid. In the opposite case, the viscous modulus ( $G''$ ) controls the  
 392 system and the material is dominated by a liquid behavior.

393 The evolution of  $\tan \delta$  is presented in **Fig. 7** and summarized in **Table 2**. In its original state,  
 394 the loss factor of the non-sonicated 12 wt% commercial suspension is inferior to 1 indicating  
 395 a dominant elastic behavior. As shown in **Fig. 5**, by applying a strong shear rate the general  
 396 viscosity decreases from 300 to 0.08 Pa·s. Upon strong deformation, the suspension quickly  
 397 recovers (200 s) its gel-like behavior with a loss of factor of 0.06.

398 **Table 2** Synthesis of  $\tan \delta$  measurements obtained with  $G'$  (elastic modulus in Pa) and  $G''$  (viscous  
 399 modulus in Pa) before and just after applying a shear rate of  $1000\text{s}^{-1}$  on 12 wt% CNC suspension

	<i>Before deformation</i>			<i>After <math>\dot{\gamma} = 1000\text{ s}^{-1}</math></i>		
	$G'$ (Pa)	$G''$ (Pa)	$\tan \delta$	$G'$ (Pa)	$G''$ (Pa)	$\tan \delta$
Non-sonicated	490	20	<b>0.04</b>	355	20	<b>0.06</b>
Sonicated	4	10	<b>2.5</b>	1	4	<b>4</b>

400 After sonication, the system is driven by the viscous modulus and presents a loss factor about  
 401 2.5. The material is dominated by a liquid behavior. After high shear rate, a loss factor value  
 402 superior to 1 is preserved.

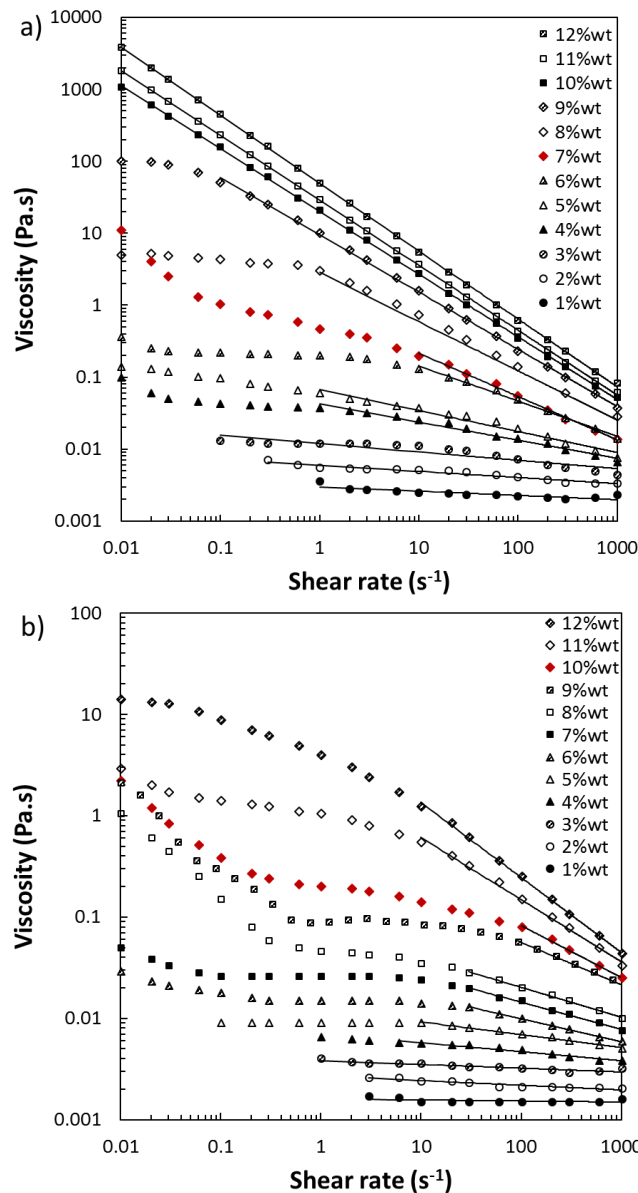
403 The rheological properties therefore changes from a gel-like behavior for unsonicated samples  
 404 to a liquid one for sonicated samples.

## 405 2.5. Concentration effect on the CNC rheological behavior

406 **Fig. 8** shows the steady-state flow curves for CNC suspensions in deionized water at different  
 407 concentrations ranging from 1 to 12 wt%. In complement, **Table 3** presents the power law  
 408 parameters obtained from fitting regions of the flow curves.

409 The behavior of CNCs without sonication treatment is shown in **Fig. 8a**. A recent study  
 410 shows results on the same type of CNCs after redispersion of a freeze-dried powder (Xu et al.,  
 411 2017b, 2018). While these results are close to our results, all concentrations were not  
 412 investigated in the publication and the impact of sonication is not evaluated. CNC suspensions  
 413 behave like near-Newtonian liquids for concentration below 3%wt, as evidenced by a flow  
 414 index  $n$  close to 1 (**Table 3**). By increasing the amount of CNCs in the suspension (4-5 wt%),  
 415 at low shear rate ( $<1\text{ s}^{-1}$ ) the CNC suspensions exhibit a viscosity plateau close to a zero-shear  
 416 viscosity plateau and at high shear rate a shear-thinning behavior ( $0.9 > n > 0.5$ ). As shown  
 417 in **Fig. 4** these concentrations correspond to the fully isotropic region. Then for 6-8%wt CNC  
 418 suspensions ( $C_i$ ), corresponding to the appearance of the liquid crystalline phase, the  
 419 rheological behavior changes to a multi-phase curve as explained by Xu et al. (Xu et al.,  
 420 2017b). The consistency  $K$  starts to increase for a 7 wt% CNC suspension ( $C_a$ ), which

421 corresponds to  $\sim 40$  vol% of liquid crystalline phase. At high shear rate, a significant shear  
 422 thinning appears with a power law exponent from 0.5 to 0.3 (**Table 3**). The slight shear-  
 423 thinning at low shear rates may indicate some association between the CNC rods. As  
 424 previously proposed (Xu et al., 2017b), at higher concentrations, which correspond to the  
 425 fully anisotropic domain, samples exhibit a strong shear-thinning behavior over the whole  
 426 range of shear rates probed. The flow index  $n$  decreased close to zero for a 12 wt% CNC  
 427 suspension, which is characteristic of a yield stress fluid. The existence of a closely packed  
 428 structure locks the rods motion due to an excessive concentration.



429

430

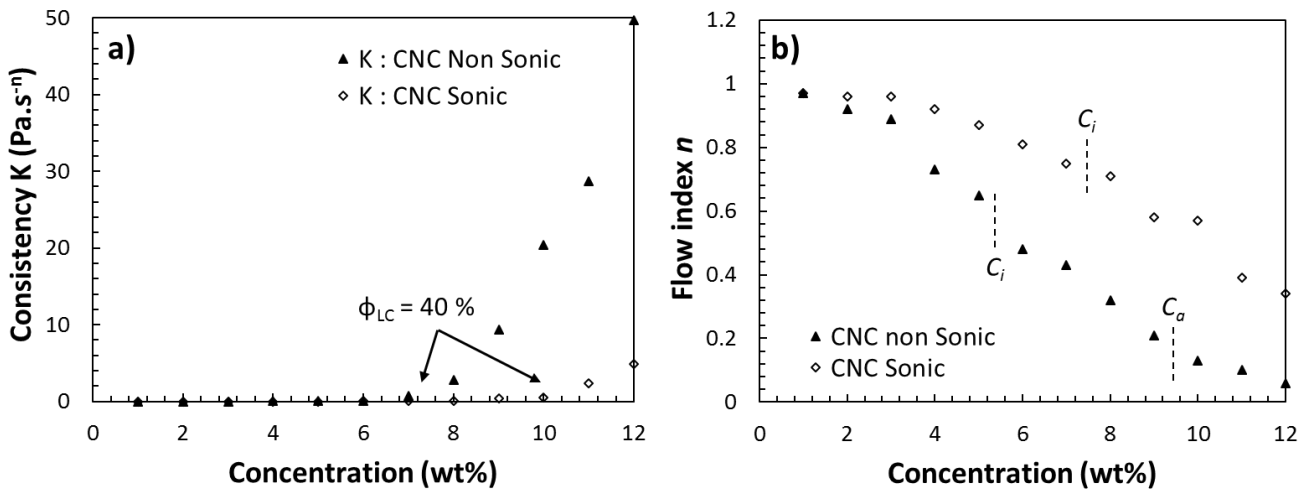
431

432 **Fig. 8** Investigation on shear rheology of 1-12 wt% CNCs suspension in water: apparent viscosity as  
 433 function of shear rate for CNC before (a) and after sonication (b). Red diamond corresponds to  $\phi_{LC}$   
 434 close to 40 vol%.

435 **Table 3** A list of the power law ( $\eta = K \cdot \dot{\gamma}^{n-1}$ ) parameters obtained from fitting regions of the flow  
 436 curves in **Fig. 8**.

		vol%	0.6	1.3	1.9	2.5	3.2	3.8	4.5	5.2	5.8	6.5	7.2	7.9
		wt%	1	2	3	4	5	6	7	8	9	10	11	12
<b>CNC Non- sonicated</b>	$K (Pa \cdot s^{-n})$	0.003	0.006	0.023	0.048	0.09	0.053	0.71	2.77	9.35	20.39	28.671	49.65	
	$n$	0.97	0.92	0.89	0.73	0.65	0.48	0.43	0.32	0.21	0.13	0.1	0.06	
	Domain	Isotropic domain						Biphasic domain			Chiral nematic domain			
<b>Sonicated CNC</b>	$K (Pa \cdot s^{-n})$	0.002	0.003	0.004	0.007	0.012	0.024	0.045	0.077	0.386	0.515	2.423	4.93	
	$N$	0.97	0.96	0.96	0.92	0.87	0.81	0.75	0.71	0.58	0.57	0.39	0.34	
	Domain	Isotropic domain						Biphasic domain						

437



438

439 **Fig. 9** Plot of the power law parameters for CNCs before and after sonication, referring to **Table 3**: a)  
 440 consistency  $K$  and b) flow index  $n$  versus CNCs concentration.

441 The rheological behavior of sonicated CNC suspensions is shown in **Fig. 8b**. According to  
 442 **Fig. 4** phase separation starts at  $C_i = 7.5$  wt% and is complete at  $C_a = 13$  wt%. CNC  
 443 suspensions at 1-4% wt exhibit a near-Newtonian behavior with  $n$  between 1 and 0.9 (**Table**  
 444 **3**). In the 5-7 wt% concentration region, at mid shear rate ( $< 10$  s<sup>-1</sup>), the CNC suspensions  
 445 exhibit a plateau region and at high shear rate a shear-thinning behavior ( $0.75 < n < 0.87$ ). A  
 446 slight shear thinning at lower shear rate could be associated to the disruption of the largest  
 447 chiral nematic domain in smaller chiral nematic domains. Then for concentrations 8, 9 and 10  
 448 wt% a three-region behavior was observed, typical of liquid crystalline samples (Bercea and  
 449 Navard, 2000; Shafiei-Sabet et al., 2012). A low shear rate ( $< 0.1$  s<sup>-1</sup>), the viscosity decrease  
 450 has been attributed to the dissociation of the largest chiral nematic domains in smaller chiral  
 451 nematic domains. Then, for middle range shear rate (1 to 30 s<sup>-1</sup>), the plateau in viscosity could  
 452 be associated to the increasing alignment of these chiral domains in the flow direction. At  
 453 high shear rate, a significant shear thinning appears and the viscosity decreases: chiral

454 domains are broken and individual CNCs will be aligned in the flow direction. For higher  
455 concentrations (11-12 wt%), the three-regions behavior is not accessible in the shear rate  
456 range probed, but a two slopes behavior persists. At  $0.1 < \dot{\gamma} < 10 \text{ s}^{-1}$ , the viscosity evolution  
457 nearly flat will correspond to organization/orientation of the nematic domains in the shear  
458 flow direction. For high shear rate ( $>10 \text{ s}^{-1}$ ), the power law parameter  $n$  decreases below 0.4  
459 and a shear-thinning behavior is observed. Nematic domains are dissociated and mostly single  
460 CNCs would aligned with the flow (Shafiei-Sabet et al., 2012).

461

462 An interesting information is emphasized in **Fig. 9a**: the consistency,  $K$ , drastically increases  
463 at 7 wt% for non-sonicated CNCs suspension and at 10 wt% for the sonicated CNCs  
464 suspension. In both cases, the volume fraction of liquid crystals deduced from **Fig. 4** is the  
465 same with an anisotropic phase volume fraction  $\Phi_{LC}$  close to 40 vol%. In **Fig. 8**, red diamonds  
466 highlight these two specific suspensions at the same  $\Phi_{LC}$  for which the rheological behaviors  
467 of non-sonicated and sonicated CNC suspension are similar. The sonication does not modify  
468 the general trend of the rheological behavior of CNC suspensions, but changes the relative  
469 concentration when the three rheological behavior regions appear. This observation highlights  
470 the fact that the suspension before sonication is not aggregated (in the case of aggregation, the  
471 general behavior is totally different (Xu et al., 2017a)) and the parameter that drives the  
472 rheological behavior is  $\Phi_{LC}$ .

473 In **Fig. 9b**, suspension of sonicated CNCs shows a power law parameter  $n$  superior to non-  
474 sonicated CNCs which corresponds to a lower shear-thinning behavior. Sonicated CNCs are  
475 well dispersed with limited constraints (due to less connection) than non-sonicated CNCs.  
476 They will flow easily thanks to minor energy needed to destruct, separate and orientate  
477 particles.

478

## 479 **2.6. Multi-scale discussion**

480 In **Fig. 4**, the as-received commercial suspension presents a phase separation behavior  
481 completed within 5-7 days, whereas longer times of the order of several weeks are usually  
482 reported for CNCs prepared from wood. This behavior proves the absence of aggregates,  
483 whose presence would not allow the self-organization to occur.

484 By comparing **Fig. 4 and 8** for both systems, it can be concluded that the rheological results  
485 present a three-regions behavior in line with the three domains observed in the phase diagram.  
486 In the fully isotropic domain, both systems present a near Newtonian nature. A plateau region  
487 appears for low shear rate. It is the beginning of the shear-thinning properties. In the biphasic  
488 domain, both systems present three rheological behavior regions as described in the literature  
489 (Ureña-Benavides et al., 2011). In the fully anisotropic region, the non-sonicated suspension  
490 is totally shear-thinning, with a shear thinning index  $n$  close to zero characteristic of a gel-like  
491 behavior.

492 This behavior might be attributed to the presence of aggregates in the CNC suspension before  
493 sonication. Some recent works have explored this hypothesis (Ureña-Benavides et al., 2011;  
494 Xu et al., 2017a, 2017b, 2018) but the authors used re-suspended freeze-dried CNCs in their  
495 studies. This process induced the formation of aggregates. In our case, however, CNC  
496 suspensions have never been freeze-dried and did not contain aggregates. As shown by AFM,  
497 TEM and DLS data (**Table 1** and **Fig. 2**) non-sonicated suspensions are composed of stable  
498 particles composed of the association of tenths of elementary crystallites into elongated  
499 bundles. Then, the gel behavior in the non sonicated CNC suspensions at high concentration,  
500 studied in this work would result from repulsive interactions of CNC particles were particles  
501 dynamics is arrested as described by Xu et al. (Xu et al., 2018). TEM, AFM, DLS and SAXS  
502 results concur to show that sonication induces a scission of the initial elongated elementary  
503 crystallites bundles into shorter and thinner objects composed of a smaller number of  
504 elementary subunits. In other words, one of the main effects of sonication is a significant  
505 decrease of the aspect ratio of the particles. Consistently and in agreement with Onsager's  
506 theory that shows that critical concentrations are inversely proportional to the aspect ratio of  
507 the particles, the phase separation behavior is shifted to higher concentrations for sonicated  
508 suspensions. However, it has to be noted that there might be other modifications induced by  
509 sonication. Beck and coworkers reported that a bound-water layer or a gel layer of sulfated  
510 oligosaccharides surrounds CNCs. They suggest that this layer contains ions originating from  
511 the hydrolysis process that might be ejected during the sonication, resulting in a larger  
512 electrical double layer and weaker chiral interactions (Beck et al., 2010, 2012). Such a  
513 mechanism would also lead to a shift towards higher concentration of the critical liquid  
514 crystalline boundaries.

515 The drastic difference in viscosity at high concentration and low shear rate illustrated in **Fig.**  
516 **1**, can also be ascribed to this aspect ratio variation. Indeed, non-sonicated high aspect ratio

517 particles logically tend to form a connected gel-like structure whereas lower aspect ratio  
518 sonicated CNCs suspensions flow due to the absence of physical interactions between the  
519 objects. As far as the rheological behavior is concerned, both non-sonicated and sonicated  
520 suspensions behave similarly and exhibit the standard rheological behavior of CNCs (Shafiei-  
521 Sabet et al., 2012; Ureña-Benavides et al., 2011) (**Fig. 8**). However, the different critical  
522 concentrations are shifted to higher values after sonication (e.g. the typical behavior of  
523 biphasic liquid crystalline suspensions appears at 7 wt% before sonication and at 10 wt% after  
524 sonication). These suspensions did not show any aggregated objects behavior and the effect of  
525 sonication is irreversible on CNCs even if some temporal variations were observed (**Fig. 6**).

526

## 527 **Conclusions**

528 The microstructure and the rheological behavior of a highly concentrated suspension of  
529 cellulose nanocrystals from University of Maine have been studied, and the behavior of never  
530 freeze-dried non-sonicated and sonicated suspensions were compared. The sonication  
531 treatment results in morphological modifications and induces a decrease of the suspension  
532 viscosity. Both suspensions present three-region phase diagrams, which correspond to three  
533 different microstructural transitions: isotropic region, biphasic region (coexistence of isotropic  
534 and chiral nematic form) and a birefringent gel. Sonication increases the critical  
535 concentrations  $C_i$  and  $C_a$  and the width of the biphasic gap and also affects the general  
536 viscosity of each concentration. At 12 wt%, the suspension is gel-like before sonication and  
537 totally turns to a liquid form after sonication treatment. The microstructure, and in particular  
538 the inter-particle distance  $d$ , is changed by this treatment. TEM, AFM, DLS and SAXS  
539 analyses consistently show that sonication induces a decrease of the aspect ratio of the  
540 particles, which can explain the observed self-organization and rheological behaviors and data  
541 rule out the presence of aggregates in the initial suspension.

542 The viscosity profile of the non-sonicated CNC suspensions shows a single shear-thinning  
543 behavior over the whole range of shear rates for concentrations higher than 9 wt%. By  
544 decreasing the concentration, a three-region behavior appears followed by a Newtonian  
545 plateau for concentrations under 3 %wt. After sonication, at high concentration, the system is  
546 stable again but presents a higher decrease of viscosity and a higher shear-thinning index. At  
547 intermediate concentrations, a notable three region plateau typical of liquid-crystal has been

548 emphasis. Then at concentrations lower than 4 wt%, the rheological behavior of the  
549 suspensions is Newtonian.

550 In this paper we have described the behavior of a commercial CNC suspension before and  
551 after sonication at high concentration. This suspension is used in many industrial applications  
552 and this study will open new opportunities for applications involving nanocrystalline cellulose  
553 like shear-thinning polymer, injectable or pump-ability liquid and matrices reinforcement.

554

555



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569

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