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# **Production of cationic nanofibrils of cellulose by twin-screw extrusion**

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## **Abstract**

Cationization of cellulose fibers introduces positives charges and thus decreases the energy consumption to produce cellulose nanofibrils (CNF), improves their quality and confers them antimicrobial properties. In this study, cationic CNF at 17 wt% solid content were produced for the first time using an energy efficient process: the twin-screw extrusion (TSE). The production was compared to the production of enzymatic CNF using the same mechanical treatment and the efficiency of cationization was discussed. CNF produced were characterized using different techniques such as optical microscopy, atomic force microscopy, mechanical properties or transparency and finally classified using the quality index. Despite a slipping effect in the TSE which leads to intact residual fibers, highly transparent CNF with high Young's modulus (10 GPa) and suitable quality index were obtained consuming only 1,100 kWh/t. Activity of cationized CNF to limit bacteria proliferation has even been confirmed.

**Keywords:** Cationization, nanofibrillation, energy consumption, antimicrobial properties

## 1. Introduction

Cellulose, the most abundant polymer on Earth, renewable, sustainable and biocompatible, is a good candidate to develop bio-based products. Cellulose nanofibrils (CNF), obtained by mechanical action on cellulose fibers present undeniable advantages. CNF possess high mechanical properties, good barrier properties and high surface area. They can be used in many applications such as packaging (Lavoine et al., 2012), painting, paper (Bardet et al., 2014; Brodin, 2014) or medicine (Jorfi and Foster, 2015). Industrial production started 8 years ago and some industrial applications are now available. CNF are already used in sport shoes, pens or care products.

To decrease the energy consumption, to facilitate the production and to increase the quality of produced CNF, cellulose fibers are submitted to chemical pretreatment before the nanofibrillation based on a mechanical process. Indeed, chemical modification of cellulose fibers allows weakening the hydrogen bonds between fibers and making the nanofibrils separation easier. Most of the time, chemically modified fibers lead to functionalized CNF of higher quality with also some additional properties. For example, phosphorylation of cellulose fibers leads to fire-retardant CNF (Ghanadpour et al., 2015). Many chemical pretreatments have been studied and very recently reviewed (Rol et al., 2018b).

Cationization of cellulose was previously reported (Abbott et al., 2006; Cai et al., 2003; Song et al., 2008, 2010; Yan et al., 2009) but its use to help the nanofibrillation is more recent. Aulin et al. (Aulin et al., 2010) were the first to cationize cellulose to make the nanofibrillation easier using 2,3-epoxypropyl trimethylammonium chloride (EPTMAC) in water, isopropanol and sodium hydroxide (NaOH) and using a microfluidizer. Then many different protocols have been proposed (Ho et al., 2011; Littunen et al., 2016; Odabas et al., 2016; Pei et al., 2013; Sehaqui et al., 2016). Different cationic reagents have been used: 2,3-epoxypropyl trimethylammonium chloride (EPTMAC) (Olszewska et al., 2011; Pei et al., 2013), and Girard's reagent T ((2-hydrazinyl-2-oxoethyl) trimethylazanium chloride, GT) (Liimatainen et al., 2014). Some studies also reported the use of solvent such as dimethylsulfoxide (DMSO), tetrahydrofuran (THF) or isopropanol to increase the cellulose modification while maintaining the crystallinity (Odabas et al., 2016). Solvent such as DMSO can be used to swell the fiber and to increase the cationic reagent penetration (Ho et al., 2011). Chaker et Boufi (Chaker and Boufi, 2015) obtained a degree of substitution (DS) between 0.05 and 0.19 by pretreating fibers in 10 wt % dimethylacetamide (DMAC) using potassium hydroxide (KOH) as a catalyst. DMAC acts as a swelling agent but also limits the hydrolysis of the epoxy ring. At a charge content of 1,160  $\mu\text{mol/g}$ , 85% of the fibers were nanofibrillated. In this study, the reaction was done in water to be the more ecofriendly and industrially available as possible. The most important parameters are the NaOH and EPTMAC quantities compared to anhydroglucose units (AGU) as well as reaction time and stirring homogeneity. Depending on the protocol DS between 0.05 (Odabas et al., 2016) and 0.74 (Song et al., 2010) were reported. High and repeatable DS are difficult to reach and it has been reported as a key parameter in the energy consumption (Saini et al., 2016). Cationization of

67 cellulose fibers, which occurs by the nucleophilic addition of the alkali-activated hydroxyl groups of  
68 cellulose to the epoxy moiety of EPTMAC (Pei et al., 2013), is a complicated modification. CNF  
69 quality depends a lot on the DS of modified fibers. With a charge content between 0.59 and 2.31  
70 mmol/g, Pei et al. (Pei et al., 2013) reported CNF with high mechanical properties, namely a Young's  
71 modulus between 9 and 10 GPa. Saini et al. (Saini et al., 2016) reported recently a diameter of 12 nm  
72 for cationic CNF instead of 62 nm for enzymatic CNF. Cellulose cationization facilitates the  
73 nanofibrillation and leads to smaller functionalized CNF of high quality. In addition to their good  
74 quality, cationic CNF present antimicrobial properties (Littunen et al., 2016; Saini et al., 2016) thank  
75 to quaternary ammonium groups which possess antiseptics and germicide properties. When the DS  
76 exceeded 0.1, cationic CNF present a bacteriostatic or a killing effect with gram positive bacteria  
77 whereas higher DS is required to have an effect on a Gram negative bacteria (Saini et al., 2016).

78 Despite chemical modification of cellulose fibers, mechanical processes traditionally used to  
79 nanofibrillate the pulp still consume a lot of energy. Saini et al. (Saini et al., 2016) reported an energy  
80 consumption between 11,000 and 3,000 kWh/t for cationic pulp with a DS between 0.05 and 0.18, by  
81 using an ultra-fine grinder. Recently, twin-screw extrusion (TSE) has appeared as an energy-efficient  
82 mechanical process to produce CNF at high solid content (Baati et al., 2017, 2018; Heiskanen et al.,  
83 2011; Ho et al., 2014; Rol et al., 2017). Enzymatic CNF at 20 wt % solid content with a high Young's  
84 modulus and a transparency of 90 % were obtained consuming 60 % less energy than an ultra-fine  
85 grinder (Rol et al., 2017).

86 The main objective of this study is to combine cationization and the energy-efficient TSE process to  
87 produce high solid content functionalized CNF, decreasing further the energy consumption and the  
88 number of passes through the TSE for preserving the quality. Indeed, for now, seven passes are  
89 required to obtain high quality CNF by TSE using enzymatic cellulose pulp (Rol et al., 2017).

## 90 **2. Material and methods**

### 91 **2.1 Materials**

92 Cellulose used throughout this work is a commercial eucalyptus bleached kraft pulp (Fibra T35,  
93 dry, moisture of  $7.00 \pm 0.13\%$ ). All the materials and chemicals were used as received from the  
94 producers: sodium hydroxide (NaOH, Roth, 98%), hydrochloric acid (HCl; Sigma Aldrich, 37%),  
95 epoxypropyl trimethyl ammonium chloride (EPTMAC, Sigma Aldrich, 90%), silver nitrate ( $\text{AgNO}_3$ ,  
96 Roth, 99%), Cellulase Fiber Care<sup>®</sup> (Novozymes, 4,500 ECU/g), acetic acid (Roth, 99%) and sodium  
97 acetate trihydrate (Sigma Aldrich, 99%). Commercial CNF from the Centre Technique du Papier  
98 (CTP, Grenoble, France) were used. Distilled water was used in solutions or suspensions  
99 preparations.

## 100 2.2 Methods

101 **Cellulose refining.** The eucalyptus bleached kraft pulp fibers were refined prior the chemical  
102 pretreatment using a pilot disk refiner (Matech Paper) equipped with 12" disks. Cellulose suspension  
103 at 2 wt % was refined until a Schopper Riegler index (°SR) of 90, measured with ISO 5267 standard.  
104 Measurements were at least done in triplicate.

105 **Cellulose Cationization.** The protocol was adapted from Saini et al. (Saini et al., 2016) previous study.  
106 40 g of refined cellulose fibers at 5 wt % were mixed with 29.6 g of NaOH diluted in 100 mL of water.  
107 The mixture is then mixed and heated during 30 min at 65°C. Finally, 4 mol/mol<sub>AGU</sub> of EPTMAC  
108 were added to the cellulose suspension and mixed for 5 h at 65°C. The reaction was done in a plastic  
109 bag placed in a heated reactor filled with water in order to be mixed easily by hand every 15 min.  
110 Finally, the suspension was neutralized using HCl, 1M and washed with water under filtration. This  
111 protocol was repeated 6 times.

112 ~~Charge content. Degree of substitution (DS). The cationic charge content DS~~ was measured by  
113 conductimetric titration. 0.4 g (dry) of modified cellulose fibers were dispersed in 200 mL of water  
114 and titrated with silver nitrate solution at 0.06 M. ~~The degree of substitution was calculated as follows.~~  
115 Titration was done at least three times and the average is presented.

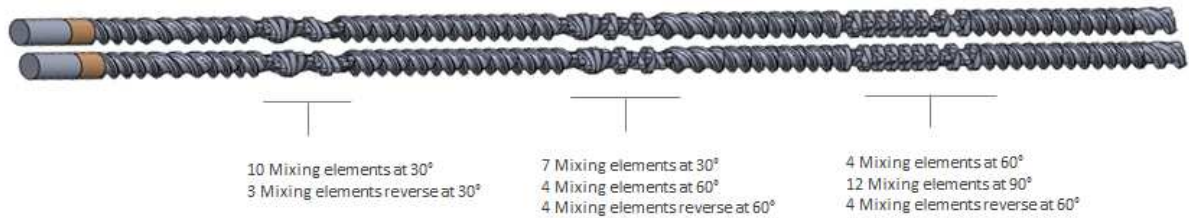
$$DS = \frac{n_{AgNO_3}}{n_{cellulose}} = \frac{C_{AgNO_3} * V_{AgNO_3 eq}}{\frac{m_{cellulose}}{M_{cellulose}}}$$

116 Where  $n_{AgNO_3}$  (mol) is the number of mole of  $AgNO_3$  at the equivalence,  $n_{cellulose}$  (mol) is the number of  
117 mole of cellulose present in the beaker,  $C_{AgNO_3}$  (mol/L) is the concentration of the solution of  $AgNO_3$ ,  
118  $V_{AgNO_3 eq}$  (L) is the volume of  $AgNO_3$  used at the equivalence,  $m_{cellulose}$  (g) is the quantity of cellulose  
119 added and  $M_{cellulose}$  (g/mol) is the molar mass of cellulose.

120 **Cellulose pretreatment with enzymes.** The eucalyptus bleached kraft pulp fibers were enzymatically  
121 treated to compare the efficiency of the cationization with the most used pretreatment at industrial  
122 scale. Cellulose fibers at 2 wt % were refined and then treated using endoglucanase enzyme solution  
123 (Fiber Care<sup>®</sup>, Novozymes). Firstly, the pH of the suspension was adjusted to 5 using a buffer of acetic  
124 acid and sodium acetate trihydrate. Then 300 ECU (EndoCellulase Units) of enzymes per gram of  
125 cellulose were added and the reaction was performed 2 h at 50°C. After killing the enzyme by heating  
126 the suspension to 90°C for 15 min, cellulose suspension was washed with deionized water under  
127 filtration and filtered to a suspension of 17 wt % solid content.

128 **Production of CNF by Twin-Screw Extrusion.** Cationic and enzymatic cellulose fibers were pressed  
129 until a solid content of 17 wt %. The fibers were then nanofibrillated using a twin-screw extruder

130 (Model Thermoscientific HAAKE Rheomex OS PTW16 + HAAKE PolyLab OS Rheodrive 7). The  
 131 temperature was maintained at 10 °C, the flow was maintained at 50 g/min (humid) and the speed was  
 132 set at 400 rpm. The screw profile is shown in Fig. 1. The cellulosic pulp was passed from one to seven  
 133 times through the TSE according to a previous study (Rol et al., 2017).



134 **Fig. 1.** Twin-screw profile used for the nanofibrillation of modified cellulose fibers

135 **Specific Mechanical Energy (SME).** Energy consumption for the production of CNF by twin-screw  
 136 extrusion was evaluated using the torque  $C$  (N.m) supplied by the software. The energy (kWh/t) is  
 137 obtained using the following equation. Calculation was done for each pass and summed up.

$$SME = \frac{N * C * P_{max}}{N_{max} * C_{max} * Q}$$

138 where  $N$  is the speed in rpm,  $N_{max}$  the maximal speed in rpm (1,100 rpm),  $P_{max}$  the maximal pressure (7  
 139 kW),  $C_{max}$  the maximal torque (130 Nm) and  $Q$  the dry flow in t/h.

140 The reported energies take into account the energy consumed by the refining step, namely 600 kWh/t.

141 **Optical microscopy.** CNF suspensions were diluted to a concentration of 0.5 wt% using an Ultra-  
 142 Turrax (1 min) stirring system. At least 10 images were taken using an optical microscope (Carl Zeiss  
 143 Axio Imager M1) equipped with an AxioCam MRC 5 digital camera and the most representatives are  
 144 presented.

145 **Atomic force microscopy (AFM).** A Dimension Icon atomic force microscope with an OTESPA  
 146 cantilever was used to take images of CNF. Firstly, CNF were diluted to a concentration of  $10^{-2}$  wt%,  
 147 then one drop was deposited on a mica disk and dried all the night at ambient temperature. At least 5  
 148 images of 5 different areas were scanned and the most representative is presented here.

149 **MorFi analysis.** The fiber length were evaluated before and after the chemical modification using a  
 150 MorFi LB-01 fiber analyzer (Techpap, France). The test was done on precisely 0.3g of dry fibers and  
 151 repeated at least two times.

152 **Nanosized fraction.** The fraction of nanofibrils in the suspension was evaluated using a method  
 153 developed by Naderi et al. (Naderi et al., 2015). CNF were redispersed to 0.02 wt% and centrifuged 15  
 154 min at 1,000 g using a centrifuge (Sigma 3-18 KS, Germany). The nanosized fraction in % is equal to

155 the suspension concentration after centrifugation against the suspension concentration before. At least,  
156 tests were done three times and the average is presented here.

157 **Turbidity.** Turbidity of diluted (0.1 wt %) CNF suspension was measured using a portable turbidimeter  
158 AL 250 T-IT (AquaLytic). The turbidimeter range is between 0.01 and 1,100 NTU. At least 10  
159 measurements were done for each sample and average is calculated.

160 **Nanopaper and Film preparation.** Films of 45 g/m<sup>2</sup> were prepared by solvent casting. 50 mL of  
161 modified CNF at 0.5 wt % were casted in Petri dish and dried in a conditioned room (25°C, 50%RH)  
162 for seven days. Nanopapers were prepared using a standard sheet former (Rapid Köthen, ISO 5269-2)  
163 equipped with a nylon sieve with a mesh size of 1 µm. 2 g of dry CNF, diluted at 1 wt % using an  
164 Ultra-Turrax were filtered and dried under vacuum in a dryer for 20 min at 90 °C. Nanopapers at 60  
165 g/m<sup>2</sup> were then stored in a conditioned room (25°C, 50%RH) for several days before characterizations.

166 **Transmittance.** Casted films transparency was evaluated using a Ultra Violet spectrophotometer  
167 Shimadzu USA MFC Inc., 70158 at 550 nm. At least 10 measurements per sample were performed.

168 **Mechanical properties.** An Instron 5965 machine equipped with a load cell of 5 kN capacity was used  
169 to measure the mechanical properties of the nanopapers at a cross-head speed of 10 mm/min.  
170 Nanopapers were first cut in rectangular specimen (100 x 15 mm). A minimum of 5 specimens were  
171 tested for each sample and the average is presented here.

172 **Simplified Quality index.** Cationized CNF quality was evaluated using the simplified quality index  
173 (QI\*) developed by Desmaisons et al. (Desmaisons et al., 2017), which involves four characterizations  
174 techniques and which is calculated as below:

$$175 \quad QI^* = 0.3 x_1 - 0.03 x_2 - 0.072 x_3^2 + 2.54 x_3 - 5.34 \ln(x_4) + 58.62$$

176 where  $x_1$  = nanosized fraction [%];  $x_2$  = turbidity [NTU];  $x_3$  = Young's modulus [GPa];  $x_4$  = macro size  
177 [ $\mu\text{m}^2$ ] measured using Image J software and optical microscopy images.

178 **Thermogravimetric analysis.** 15 mg of modified CNF were placed in a thermogravimetric analyzer  
179 (TA Instrument Q500), heated from 30°C to 600°C with a heating rate of 10°C.min<sup>-1</sup> under O<sub>2</sub>  
180 atmosphere and finally pyrolyzed from 600°C to 900°C at 50 °C.min<sup>-1</sup>. The apparatus recorded the  
181 mass loss as a function of temperature. Duplicate were performed.

182 **Antimicrobial properties.** Antibacterial activity against *S. aureus* and *E. coli* was tested using the  
183 AATCC 100-1998 Test Method under static conditions. Quantitative assessment of the antibacterial  
184 activity was measured. 200 µL of inoculum was placed on each film sample surface (50 mg) (modified

185 or not). After 24h of incubation at 37°C, 50 mL of neutralizing solution were used to extract the  
186 bacteria and they were plated on nutrient agar using pour plate method. Finally, bacteria were  
187 incubated 24h at 35°C and the number of colonies forming units (CFU) was counted using pour plate  
188 method. The antibacterial activity of cationized nanocellulose films was calculated using the following  
189 equation:

190 
$$\text{Log reduction} = \text{Log CFU T24 untreated sample} - \text{Log CFU T24 treated sample}$$

191 At least triplicates were performed for each sample.



## 192        **2. Results and discussions**

193        Cellulose fibers were cationized and high DS are expected to decrease the energy consumption  
194 (Saini et al., 2016). Finally, cationization and twin-screw extrusion (TSE) were combined to try to  
195 decrease the energy consumption preserving the high quality of cationic CNF produced with  
196 traditional mechanical processes. Degree of substitution (DS) of modified cellulose fibers was  
197 measured by **conductimetric titration** and equal to  $0.31 \pm 0.08$ . Due to the high quantity of cellulose  
198 materials required by the extruder, four different batches of cellulose cationization were done and two  
199 batches with approximately the same DS were mixed and passed through the TSE. High DS were  
200 obtained (Saini et al., 2016) but poor repeatability was observed comparing the four batches.

201        Many parameters influence the reaction and make it difficult to reproduce. However, the same  
202 NaOH and EPTMAC charges and the same time and temperature were used, meaning that the main  
203 problem resides in the stirring homogeneity of the high quantity of cellulose fibers required for the  
204 nanofibrillation step using TSE, (i.e. about 200 g dry). Homogenization of such a quantity by using the  
205 classic plastic bag reactor at this concentration is complicated and leads to heterogeneity in the surface  
206 modification.

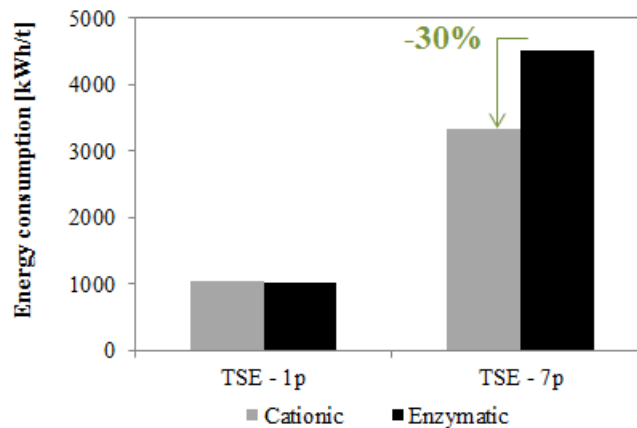
### 207        **3.1 Cationization and TSE: an efficient combination to produce CNF**

208        Despite several very recent papers regarding cationic CNF production, none of them reported the  
209 use of this pretreatment and TSE to produce functionalized cellulose nanofibrils. Moreover, only few  
210 publications deal with a complete characterization of cationic CNF.

#### 211        **3.1.1 Energy saving using this combination**

212        CNF at 17 wt % solid content were produced using a TSE and enzymatic hydrolyzed or cationized  
213 cellulose pulps. Different grade of CNF were produced by passing the pulp from 1 to 7 times through  
214 the TSE. The energy consumptions to produce such CNF are reported in Fig. 2 and take into account  
215 the similar energy consumed by the refining step, namely 600 kWh/t.

216        Cationization of cellulose allows decreasing a lot the energy consumption after 7 passes through the  
217 TSE compared to the enzymatic pretreatment. This result demonstrates the efficiency of the  
218 introduction of positive charges onto cellulose fibers. The first pass in the TSE consumes almost the  
219 same energy for both pretreatments whereas a decrease of 30% is observed after 7 passes. Between 10  
220 and 30 % of reduction were observed from passes 2 to 7. Saini et al. (Saini et al., 2016) also reported a  
221 decrease of 36% in the energy consumption by increasing the degree of substitution from 0.05 to 0.18  
222 using an ultra-fine grinder. Cationization introduces positive charges onto the surface of cellulose  
223 nanofibrils, increases the repulsion between fibrils, decreases the number of H-bonds and hence makes  
224 the fiber separation easier. Moreover, some fiber swelling happens (Pei et al., 2013) due to the  
225 polysaccharides solvation with NaOH which might help the nanofibrillation.



226 **Fig. 2.** Energy consumption and saving for the nanofibrillation of cationic cellulose instead of  
 227 enzymatic hydrolyzed fibers by TSE

228 Nanofibrillation is easier and energy consumed is decreased compared to enzymatic cellulose,  
 229 making the industrialization of such CNF more probable. However, the CNF quality should be  
 230 discussed before concluding. To not overcrowd the study, only the CNF suspensions obtained after 1  
 231 and 7 passes through the TSE are characterized. Indeed, in a previous study, it has been already shown  
 232 that no more evolution occurs after 7 passes (Rol et al., 2017). Ho et al. (Ho et al., 2014) reported no  
 233 change after 10 passes for non-pretreated cellulose fibers.

### 234 3.1.2 Impact of the TSE on the pretreated fibers morphologies

235 Modified fibers before the nanofibrillation were analyzed using a MorFi analyzer. Whereas the non-  
 236 pretreated refined fibers present a fiber length of  $600 \pm 10 \mu\text{m}$ , the modified fibers have length of  $544$   
 237  $\pm 6$  and  $400 \pm 10 \mu\text{m}$  for cationic cellulose fibers and enzymatic cellulose fibers respectively. The  
 238 enzymatic pretreatment considerably decreases the fibers length whereas the cationization does not  
 239 affect the fibers length. This will probably impact the nanofibrillation efficiency.

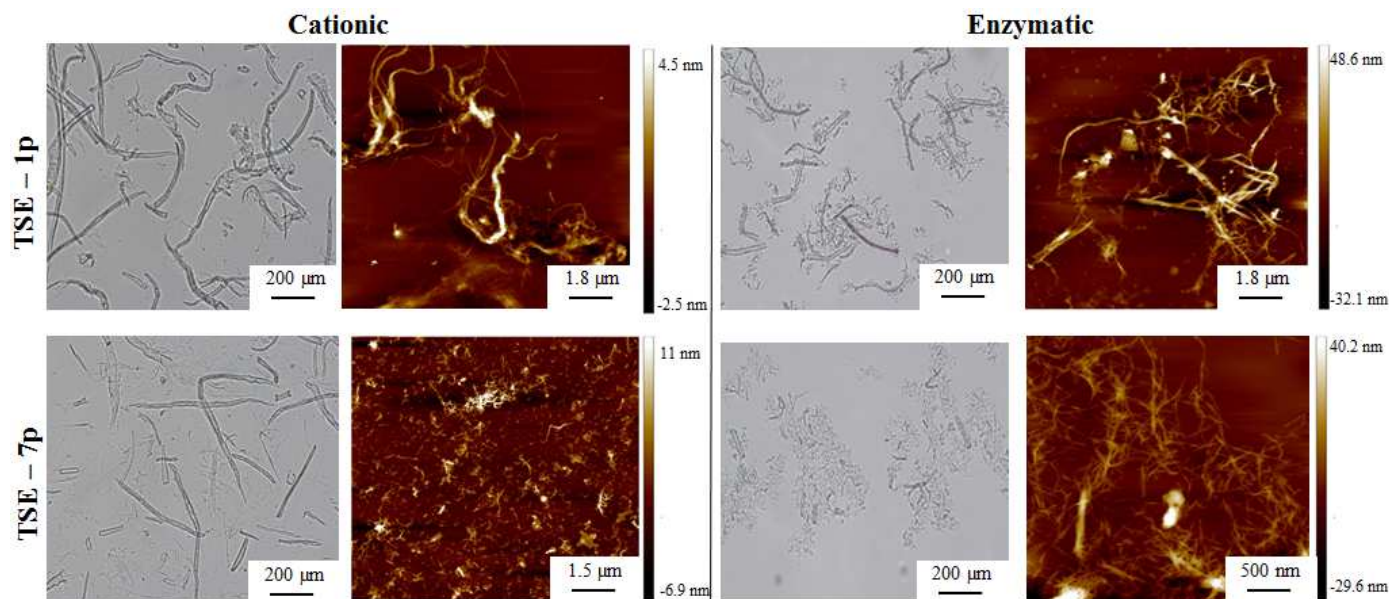
240 Morphologies of produced CNF have been observed using optical microscopy and AFM images as  
 241 shown in Fig. 3.

242 Big residual fibers are present in the cationic CNF suspensions after 1 or 7 passes through the TSE  
 243 whereas enzymatic CNF suspension does not contain any fibers after 7 passes. Due to the high  
 244 viscosity of the cationized pulp, there might be a slipping effect of fibers into the TSE and intact big  
 245 fibers remain into the suspension. The issue of heterogeneous grafting during cationization, or the  
 246 higher length of cationic fibers might also explain such results, with some fibers with lower DS. This  
 247 phenomenon has already been reported for TEMPO-oxidized cellulose pulp (Rol et al., 2017). Insofar  
 248 as residual fibers do not present any sign of fibrillation, it confirms their slipping among the screws.  
 249 Indeed, even when cellulose fibers were not chemically treated, fibers passing through the TSE  
 250 always present a certain level of fibrillation (Rol et al., 2017). Residual fibers were also reported for a  
 251 suspension with a DS of 0.18 passed through an ultra-fine grinder (Saini et al., 2016). However, fibers

252 content tends to decrease with the number of passes through the TSE for both pretreatments,  
253 demonstrating the TSE efficiency as reported previously (Rol et al., 2017, 2018a).

254 The macro size, used for the calculation of the simplified quality index and presented in Table 1,  
255 represents the surface of residual fibers visible on optical microscopy images. Cationic CNF present  
256 residual fibers explaining the higher macro size than that of enzymatic CNF. However, the decrease in  
257 the macro size with the number of passes confirms the efficient nanofibrillation. To conclude, the  
258 quality of produced CNF will be negatively impacted by the presence of those residual fibers and the  
259 characterization techniques used later can be skewed.

260 Despite these residual fibers, nanometric fibers are also observed by AFM for each suspension after  
261 1 or 7 passes. Agglomerates and bigger nanofibers were observed for the first passes, confirming the  
262 efficient nanofibrillation with the number of passes through the TSE. Size measurement is complicated  
263 on those images due to the agglomeration and entanglements but an average diameter of  $65 \pm 30$  nm  
264 was measured for both pretreatments after 1 pass. The high standard deviation confirms the  
265 heterogeneity of the suspensions. Nanofibers of different sizes are also observed after 7 passes: width  
266 of  $52 \pm 21$  and  $43 \pm 20$  nm are measured for enzymatic CNF and cationic CNF respectively,  
267 confirming that chemical modification of fibers surface leads to slightly smaller CNF. Indeed,  
268 according to the literature, most of the time, charged CNF such as TEMPO-oxidized or cationic,  
269 contain smaller and more homogeneous nanofibers than enzymatic CNF (Limatainen et al., 2013;  
270 Nechyporchuk et al., 2015; Olszewska et al., 2011; Saito and Isogai, 2006; Sirviö et al., 2015).  
271 Cationic nanofibrils become thinner and more homogeneous when the modification rate increases until  
272 a DS of 0.37 (Pei et al., 2013). The results are in agreement with Chaker and Boufi (Chaker and  
273 Boufi, 2015), who reported a width between 20 and 40 nm for cationic CNF. Cationic CNF and  
274 enzymatic CNF have apparently similar morphology but it seems that individual cationic CNF are  
275 smaller than enzymatic ones due to fiber repulsion which facilitates the nanofibrillation and hence  
276 lead to smaller CNF.



277 **Fig. 3.** Mor phology of produced CNF by TSE observed using optical microscopy and AFM

278 Optical techniques are a good way to visualize the suspensions morphologies but they are not  
 279 representatives of the total suspension. This is why additional techniques were used.

280 Turbidity of CNF suspensions was also used to characterize CNF morphology and results are  
 281 reported in Table 1. Cationic CNF suspensions present much smaller turbidity than enzymatic CNF  
 282 ones, whatever the number of passes, confirming the smaller size of individual cationic CNF and /or  
 283 the higher dispersion due to the repulsion with positive charges. Passing 1 time the cationic pulp  
 284 through the TSE leads to CNF with lower turbidity than after 7 passes for enzymatic pulp, confirming  
 285 the efficiency of cationization to decrease the number of passes through the TSE. However, contrary to  
 286 enzymatic fibers, no evolution was reported with the number of passes through the TSE for the  
 287 cationic fibers, meaning that no more nanofibrillation occurs after the first pass, due to the slipping  
 288 effect. Finally, reported data are in agreement with the literature : 242 NTU for enzymatic CNF treated  
 289 via an ultra-fine grinder (Desmaisons et al., 2017) and 19.8 NTU for TEMPO-oxidized CNF via a  
 290 homogenizer (Moser et al., 2015) have been reported.

291 **The measure of the nanosized fraction, detailed in Materials and methods,** of the CNF suspensions  
 292 is the last method used to evaluate the CNF **suspension composition morphology.** Results are reported  
 293 in Table 1 but due to high standard deviation, comparisons are difficult. Nanosized fraction increases  
 294 with the efficient nanofibrillation of the enzymatic pulp passed from 1 to 7 times through the TSE.  
 295 Once again, no evolution is reported for the cationic pulp which can be easily explained by the  
 296 presence of residual fibers which settle down during the centrifugation step or by the absence of  
 297 efficient nanofibrillation. After 7 passes for the cationic pulp, the nanosized fraction obtained is closed  
 298 to the one of the enzymatic CNF suspension obtained after one pass. Cationization does not help to  
 299 reduce the number of passes. However, Naderi et al. (Naderi et al., 2015) reported similar nanosized

300 fraction for carboxymethylated CNF obtained with a homogenizer which means that the suspensions  
301 quality is pretty good.

302 Cationic CNF suspensions are composed of a mix of big intact residual fibers, nanofibrils  
303 aggregates and very small individual nanofibers, smaller than enzymatic CNF after 7 passes. Due to  
304 the slipping effect in the TSE, no evolution in the suspension morphology is clearly observed with the  
305 number of passes for this modified pulp. This can express a non-nanofibrillation or merely that the  
306 residual fibers influence too much the characterization techniques. From a morphological point of  
307 view, cationization does not allow reducing the number of passes through the TSE compared to  
308 enzymatic pulp or to reach higher quality. Other characteristics than morphological one will now be  
309 tested.

### 310 **3.1.3 Quality of the functionalized CNF produced by TSE**

311 Characterizing CNF and evaluating their quality is still challenging today (Foster et al., 2018;  
312 Kangas et al., 2014). Different techniques can be used and a quality index (QI or a simplified one  
313 QI\*), which involves some of them has been recently proposed to easily characterize and classify CNF  
314 (Desmaisons et al., 2017). Nevertheless, the QI, developed for enzymatic CNF, is maybe not fully  
315 adapted to functionalized CNF. It gives mainly an indication about homogeneity of CNF and not really  
316 the quality. For 1 pass, QI\* for cationic CNF ( $QI^* = 60.9 \pm 3.0$ ) was slightly higher than that of  
317 enzymatic CNF ( $QI^* = 57.4 \pm 0.2$ ). When the number of passes increases until 7, there is no change of  
318 QI\* for cationic CNF ( $60.7 \pm 0.4$ ) whereas a clear improvement is observed for enzymatic CNF ( $QI^*$   
319  $= 69.5 \pm 1.8$ ). This can be easily explained by the lower nanofibrillation yield, due to the slipping  
320 effect of cationic cellulose fibers and hence by the presence of residual fibers which influences  
321 negatively many characterizations tests. It seems that only 1 pass is sufficient for cationic CNF or at  
322 least no clear improvement is observed. Nanofibrillation can be stopped after 1 pass, which results in  
323 lower energy consumption.

324 It is then important to consider more in details the mechanical properties of nanopapers. The  
325 Young's modulus, toughness and the tensile strength are reported in Table 1. Such characterization of  
326 cationic nanopaper was poorly reported in the literature (Pei et al., 2013). Mechanical properties are  
327 affected by several parameters such as porosity of the nanopaper, intrinsic properties of the nanofibers  
328 and the number of hydrogen bonds between fibrils (González et al., 2012, 2014; Kulachenko et al.,  
329 2012). Cationic CNF present a Young's modulus between 7 and 10 GPa, depending on the number of  
330 passes whereas enzymatic CNF present higher Young's modulus (between 10 and 12 GPa). After the  
331 first pass through the TSE, both suspensions present equivalent Young's modulus. The residual fibers,  
332 which are less rigid and can create privileged failure zones, do not influence mechanical properties  
333 because of high interaction between the matrix made of CNF and the filler constituted by the residual  
334 fibers (González et al., 2014). The CNF quantity, namely around 54% as measured by the nanosized  
335 fraction, is sufficient to obtain high mechanical properties. Moreover, higher tensile strengths are

336 obtained for cationic CNF, probably due to the higher entanglement and number of hydrogen bonds or  
 337 ionic interactions. The higher cellulose DP of the cationic CNF, presented Table 1, can also influence  
 338 the tensile strength and toughness (Henriksson et al., 2008).

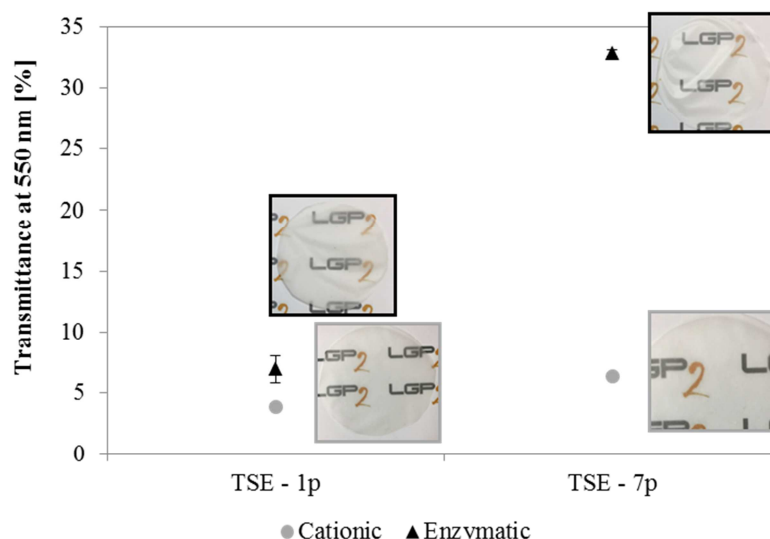
339 The resistance of both cationic and enzymatic cellulose nanopapers to fracture were measured and  
 340 reported in Table 1. Cationic CNF present much higher toughness than enzymatic CNF but no  
 341 evolution is reported with the number of passes through the TSE. Cationic CNF present smaller size  
 342 and higher cohesion and thus lead to higher resistance. Data reported here are slightly lower than the  
 343 ones reported in the literature. Indeed toughness between 6.5 (Mao et al., 2017) and 15.1 (Henriksson  
 344 et al., 2008) MJ/m<sup>3</sup> were reported for enzymatic CNF.

345 **Table 1.** Evaluation of CNF quality using the simplified quality index

Pretreatment	Mechanical processes	Macro size by			Young's modulus [GPa]	Tensile strength [MPa]	Toughness [MJ/m <sup>3</sup> ]	Cellulose DP
		optical microscopy [μm <sup>2</sup> ]	Nanosized fraction [%]	Turbidity [NTU]				
Cationic	TSE - 1p	185 ± 84	50.6 ± 10.0	113 ± 4	9.6 ± 0.5	74.1 ± 0.6	3.50 ± 0.30	721 ± 3
	TSE - 7p	131 ± 59	54.6 ± 2.5	113 ± 6	7.3 ± 1.0	72 ± 2.9	3.20 ± 0.90	731 ± 3
Enzymatic	TSE - 1p	75 ± 33	47.1 ± 10.3	366 ± 44	9.8 ± 0.3	32.7 ± 12.7	0.22 ± 0.07	324 ± 2
	TSE - 7p	41 ± 12	64.3 ± 9.9	289 ± 13	11.6 ± 0.3	22 ± 8	0.17 ± 0.03	218 ± 1

346 Whereas mechanical properties increase with the efficient nanofibrillation for the enzymatic CNF,  
 347 no evolution was observed for the functionalized CNF, confirming that no more nanofibrillation  
 348 occurs after the first pass. After 7 passes for the cationized pulp, similar Young's modulus as after 1  
 349 pass for the enzymatic pulp are obtained.

350 Transmittance of cationic films has been measured on casted films and reported in Fig. 4.  
 351 **Transmittance allows to compare the samples and to follow the evolution with the number of passes**  
 352 **but does not correspond exactly to the transparency. Despite low transmittance, highly transparent**  
 353 **films were obtained for both pretreatments, as observed in images in Fig. 4.** However enzymatic CNF  
 354 present higher transmittance whatever the number of passes. Once again, slight evolution of the  
 355 transparency is reported for cationic pulp confirming that further nanofibrillation after 1 pass is not  
 356 efficient. CNF morphologies also influence the transmittance of CNF films. Enzymatic CNF presents  
 357 higher transmittance due to the absence of residual fibers which created pores and increase the  
 358 roughness of the film, decreasing the transmittance. Finally, cationic films are transparent due to the  
 359 very small diameters of nanofibrils and such transparency is enough for most of the CNF application.



**Fig. 4.** Evaluation of the transparency of films of cationic and enzymatic CNF produced via different number of passes through the TSE

Cationization of cellulose fibers does not allow reaching similar  $QI^*$  than enzymatic pulp at high number of passes through the TSE. Anyway, suitable  $QI^*$  are obtained for cationic CNF after only 1 pass through the TSE, consuming only 500 kWh/t or 1,100 kWh/t taking into account the refining step, which is very low compared to the literature (Rol et al., 2017; Spence et al., 2011). Such  $QI^*$  are sufficient for numerous applications of CNF such as in packaging for example. Moreover, the cationic CNF present additional properties, such as antimicrobial properties, compared to enzymatic CNF as exposed in the following part.

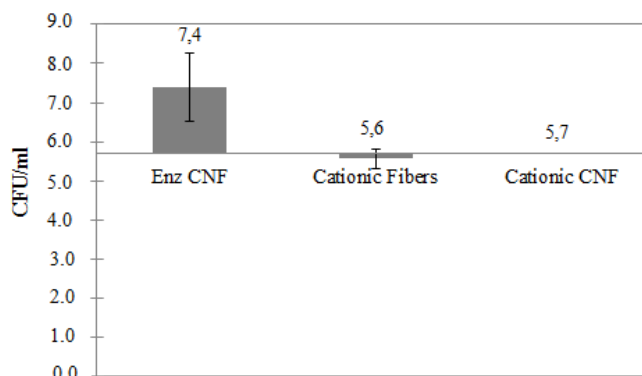
### 3.2 Antimicrobial properties of the cationized CNF

Contact bactericidal activity of cationized CNF was tested using the AATCC 100 test method as reported in Fig. 5. The reference used is enzymatic commercial CNF. The antimicrobial effect is due to the presence of quaternary ammonium compounds (Roy et al., 2008), which interact with the negatively charged polycations of phospholipids and proteins present on the surface of bacterial cells. That is why no influence in the antibacterial activity was reported between cationic fiber and cationic CNF. When quaternary ammonium compounds and the polycations interact, the cytoplasmic membrane permeability of the bacteria is damaged and there is some loss of cytoplasmic constituents. This loss causes the bacteria death (Denyer, 1995; Roy et al., 2008).

The antibacterial effect of the produced cationic cellulose was tested against Gram negative *E.coli* and Gram positive *S.aureus* bacteria. Enzymatic CNF present a bacterial growth of 7.4 CFU/mL when films were put in contact with *S.aureus* and have no bactericidal effect. As expected, no contact antibacterial effect was reported for Gram negative *E.coli* bacteria (results not shown) whereas bacteriostatic effect was reported for Gram positive bacteria as shown in Fig. 5. Indeed, quaternary ammonium is less effective against Gram negative bacteria (Sun et al., 2006) and higher DS are



384 required to kill this type of bacteria (Ladhari et al., 2007). Saini et al. (Saini et al., 2016) also reported  
385 a growth of three logs whatever the DS of the cationic CNF. To conclude, higher DS are required to  
386 have a more significant impact on the antimicrobial properties (Saini et al., 2016) but for now  
387 produced cationic CNF can be used in preventing some bacteria proliferation.



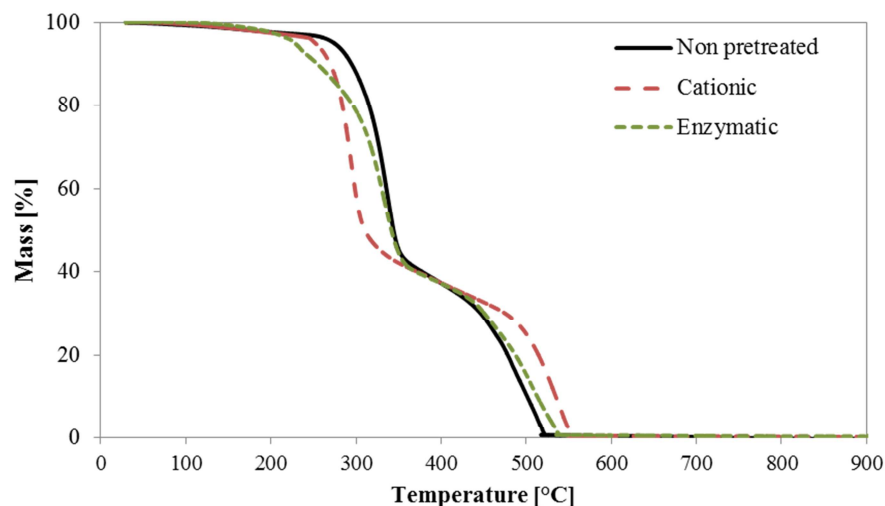
388 **Fig. 5.** Evaluation of antimicrobial properties of cationic cellulose fibers and nanofibers against  
389 Gram positive *S.aureus* bacteria

390 Finally, chemical pretreatment of cellulose fibers and mechanical processes used for the  
391 nanofibrillation always lead to some cellulose degradation. The impact of cationization on the fibers is  
392 evaluated in the following part.

### 393 **3.3 Cellulose degradation caused by the cationization and the TSE**

394 Thermal degradation and degree of polymerization of cellulose of cationic CNF were measured in  
395 order to evaluate the cellulose degradation during the cationization step and are presented in Fig. 6 and  
396 Table 1. Cationic CNF present slightly lower degradation temperature (-35°C) than enzymatic CNF as  
397 reported in the literature (Sehaqui et al., 2016; Yan et al., 2009; Zhang et al., 2015), meaning that  
398 cationization decreases the thermal stability of modified cellulose. **The degradation temperatures were**  
399 **evaluated using the derivative weight evolution in function of the temperature recorded during the**  
400 **analysis. However, the change in the degradation profiles between cationic and enzymatic pulps can**  
401 **also be due to the potential degradation of the ammonium groups present on cationic fibers or due to**  
402 **the difference in crystallization degrees or fiber morphologies (Zhang et al., 2015).**





403 **Fig. 6.** Study of the cellulose fibers degradation by the cationization: effect on thermal stability

404 Degree of polymerization of cellulose of nanofibers was measured and presented in Table 1.  
 405 However, the method used to calculate the cellulose DP is dedicated to pure cellulose and paper pulps.  
 406 Results should be taken with caution insofar as cellulose fibers are chemically modified. Cellulose DP  
 407 is well known to decrease due to mechanical treatment (Ho et al., 2014; Iwamoto et al., 2007) and  
 408 chemical treatments (Henriksson et al., 2007; Isogai et al., 2011; Shinoda et al., 2012). Whereas  
 409 enzymatic pretreatment decreases the cellulose DP from 1,200 to 600 due to enzymes which attack the  
 410 amorphous domain, cationization slightly decreases the cellulose DP from 1,200 to 1,130. However,  
 411 Pei et al. (Pei et al., 2013) reported that cationization also attacks the amorphous domains of cellulose  
 412 fibers resulting sometimes in their dissolution. Here cellulose DP is not affected too much maybe due  
 413 to a heterogeneous modification and the presence of non-modified fibers or a non-fully adapted  
 414 measurement method.

415 TSE as other mechanical processes decreases the cellulose DP of CNF and conventional cellulose  
 416 DPs were obtained for enzymatic CNF (Henriksson et al., 2007; Rol et al., 2017) whereas cationic  
 417 CNF presents really high cellulose DP, which can be easily explained by the presence of residual  
 418 fibers which overestimate the viscosity.

419 Cationization such as other chemical pretreatments leads to small decrease in the thermal stability  
 420 of the cellulose and no change in the crystallinity index was reported in the literature (Pei et al., 2013;  
 421 Saini et al., 2016). Degree of polymerization of cellulose of CNF is normally decreased for a  
 422 homogeneous modification of cellulose fibers but is slightly affected here.

## 423 **Conclusion**

424 Cationic CNF are promising but their use at industrial scale is compromised due to the lack of  
 425 homogeneity and reproducibility of such chemical modification. In this study, cellulose fibers  
 426 cationization and TSE were then combined to produce high quality CNF, decreasing the energy

427 consumption and the number of passes through the TSE compared to enzymatic CNF. High solid  
428 content (17 wt %) cationic CNF with a DS of 0.31 were hence produced for the first time and an  
429 energy consumption decrease of 30% was reported after 7 passes compared to the production of  
430 enzymatic CNF. Unfortunately, the cationization does not allow reducing the number of passes nor  
431 increases the quality of produced CNF, due to the heterogeneous chemical modification of fibers and  
432 their slipping into the TSE. However, cationic CNF with high transparency and high Young's modulus  
433 were produced in one TSE pass, consuming 1,100 kWh/t as total energy. Moreover, thanks to the  
434 presence of quaternary ammonium, modified CNF present bacteriostatic properties against Gram  
435 positive bacteria and can be used to limit the bacteria proliferation in various applications such as food  
436 packaging or medicine.

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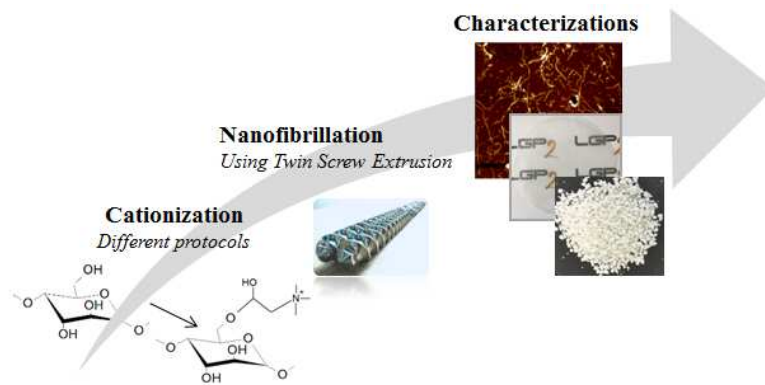
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**Graphical abstract:** Schematic illustration of this study about the production of cationic CNF by twin-screw extrusion