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1

2 **Controlled hydrophobic modification of cellulose nanocrystals for tunable**

3 **Pickering emulsions**

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7

8 **Abstract**

9 Cellulose nanocrystals (CNC) are known to promote highly stable oil-in-water (O/W)
10 Pickering emulsions. Our work consisted in producing CNCs with different
11 hydrophilic/hydrophobic balance at their surface by chemical functionalization (acylation
12 with model functional vinyl esters) and to identify the adequate conditions allowing the
13 stabilization of direct O/W or inverse W/O Pickering emulsions. Whatever the surface degree
14 of substitution (DS_{Surf}), CNCs grafted with linear acyl chains of 2 to 6 carbons led to the
15 exclusive formation of direct O/W emulsions. Distinctively, both O/W and W/O emulsions
16 could be obtained when the linear chain contained 8 carbons or more, at low and high DS_{Surf} ,
17 respectively. By adjusting the length of the grafted chain, DS_{Surf} and particles concentration,
18 we were able to monitor the type of emulsion formed, droplet size and surface coverage at the
19 oil/water interface.

20 **1. Introduction**

21 An emulsion is a system of dispersed droplets of one immiscible liquid in another,
22 stabilized by an amphiphilic surface reactive agent composed by both a polar hydrophobic
23 and a non-polar hydrophilic extremities. Emulsions are being used in a broad range of daily
24 life products, such as in food industry, cosmetics, or pharmaceuticals, and in most cases, the
25 surface active agents used are surfactants; i.e. chemical products. However, surfactants may
26 be the cause of some undesirable adverse effects, such as skin irritation for the consumer, but
27 also have negative impact on the environment (Belanger et al., 2006). As an alternative to
28 chemical surfactants, emulsions can also be stabilized by the use of solid particles, forming
29 so-called Pickering emulsions (Pickering, 1907; Arditty, Whitby, Binks, Schmitt, & Leal-
30 Calderon, 2003). This type of emulsion also has the advantage of having an adsorption energy
31 at the interface thousands times greater than for surfactants, leading to a better stability of the
32 emulsion with the particles irreversibly anchored at the interface of the emulsion (Binks,
33 2002). Among the most interesting solid particles used for Pickering emulsions are cellulose
34 nanocrystals (CNCs). Indeed, cellulose is a biodegradable biopolymer, main building block of
35 trees and plants and composed of ordered crystalline regions interrupted by amorphous
36 regions. The amorphous regions can be hydrolyzed using sulfuric acid, resulting in the
37 production of rod-like nanocrystals decorated with sulfate half-ester groups at their surface
38 (Habibi, Lucia, & Rojas, 2010; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011). The
39 use of unmodified CNCs as Pickering emulsion stabilizer has been first demonstrated in 2011
40 (Kalashnikova, Bizot, Cathala, & Capron, 2011). It was shown that unmodified CNCs
41 dispersed in water, mixed with an apolar phase, such as hexadecane, can adsorb at the oil-
42 water interface, preventing destabilization from coalescence for a very long period of more
43 than one year. Small angle neutron scattering experiments carried out with a contrast variation
44 method probed the arrangement of CNCs at the interface. These results showed that the (200)

45 crystalline plane of the CNCs directly interacts with the interface without deforming it
46 (Cherhal, Cousin, & Capron, 2016). As a consequence, surface interactions occur between the
47 CH of the CNCs and the alkyl chain of hexadecane after desorption of the aqueous layer.
48 These performances in stabilizing oil-in-water (O/W) Pickering emulsions are reached
49 through the formation a dense 2D interfacial network around the droplets (Kalashnikova,
50 Bizot, Cathala, & Capron, 2012), with the possibility to control their size by varying the
51 CNCs concentration (Capron, Rojas, & Bordes, 2017; Cherhal, Cousin, & Capron, 2016).
52 This process generally requires the addition of salt to screen the repulsion between the
53 negatively charged particles. In another approach, surface modification can also be carried out
54 (Eyley & Thielemans, 2014; Habibi, 2014), to finely adjust the hydrophilic/hydrophobic
55 balance at the surface of the CNCs. In that case, O/W (Sèbe, Ham-Pichavant, & Pecastaings,
56 2013; Werner, Schmitt, Sèbe, & Héroguez, 2017; Werner, Sèbe & Héroguez, 2018), water-in-
57 oil (W/O) (Xhanari, Syverud, & Stenius, 2011; Lee, Blaker, Murakami, Heng, & Bismarck,
58 2014; Zhang, Tam, Wang, & Sèbe, 2018; Werner, Schmitt, Sèbe, & Héroguez, 2019) or
59 double (Cunha, Mougél, Cathala, Berglund, & Capron, 2014) Pickering emulsions can be
60 obtained, depending on the surface wettability of the particles.

61 In this work, a wide range of amphiphilic cellulose nanocrystals were prepared by
62 acylation with model vinyl esters of various chain length and surface degree of substitution
63 (DS_{Surf}), in order to i) investigate the impact of the surface functionalization on the properties
64 of Pickering emulsions prepared from hexadecane, and ii) identify the adequate conditions
65 allowing the stabilization of O/W or W/O emulsions.

66 2. Material and methods

67 2.1 Material

68 Cellulose nanocrystals (CNCs) were isolated by sulfuric acid treatment of wood pulp and
69 purchased from the University of Maine. Vinyl esters (in brackets: nomenclature, degree of
70 purity) were purchased from Sigma Aldrich or TCI suppliers and not further purified before
71 use: vinyl acetate (VA, 99%, Sigma), vinyl propionate (VPro, 98%, Sigma), vinyl butyrate
72 (VBut, 98%, TCI), vinyl hexanoate (VHex, 99%, TCI), vinyl n-octanoate (VOct, 99%, TCI),
73 vinyl decanoate (VDec, 99%, TCI), vinyl laurate (VLau, 99%, TCI), Vinyl myristate (VMyr,
74 99%, TCI), vinyl palmitate (VPalm, 96%, TCI) and vinyl stearate (VSte, 95%, TCI).
75 Potassium carbonate (K_2CO_3), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), toluene,
76 hexadecane, fluorescein, technical acetone and ethanol were purchased from Sigma Aldrich.
77 DMSO and K_2CO_3 were systematically dried beforehand: on 5-Å molecular sieve for DMSO;
78 in an oven at 50°C for K_2CO_3 (overnight).

79 2.2 Chemical functionalization of the CNCs

80 After weighing 2.5 g of CNCs and 1.0 g of K_2CO_3 in a double-necked 150 mL flask,
81 100 mL of dried DMSO was added and the solution was sonicated for 1 min (Bandelin, MS72
82 probe, 1.5 kJ). The volume of vinyl ester added was calculated for each treatment, to fit the
83 molar ratio $N_{\text{vinyl ester}}/N_{\text{OHs}} = 3.75$, with N_{OHs} corresponding to the number of OH groups
84 available at the surface of the CNCs (estimated at 3.1 mmol.g⁻¹ in Brand, Pecastaings, &
85 Sèbe, 2017). After introduction of the vinyl ester, the mixture was heated in an oil bath
86 sustained at 80°C, and magnetically stirred at 250 rpm for 5h. The reaction medium was then
87 sampled at different periods of time (10 min, 15 min, 30 min, 45 min, 1h, 2h, 3h, 4h or 5h).
88 Each sample was quenched with THF, and then cooled down in a water bath until room
89 temperature was reached. Any remaining reagent or organic by-products was removed by
90 several successive washing/sedimentation steps using 1) THF, 2) ethanol and 3) acetone, and

91 with the help of a centrifuge. The modified particles were then dialyzed in water for two days
92 using a 1 kDa regenerated cellulose membrane and changing the water bath two times a day,
93 to remove the K_2CO_3 catalyst. Finally, the particles were re-dispersed by sonication and
94 freeze-dried.

95 *2.3 Fourier Transform Infrared Spectroscopy (FT-IR)*

96 1-2 wt. % of modified CNC was grinded in a KBr matrix, then pressed under 200 bars for
97 5 min to form a pellet. The spectra were recorded on a Fourier Transform Infrared Bruker
98 spectrometer in transmission mode. Spectra were recorded between 4000 and 400 cm^{-1} , with a
99 resolution of 4 cm^{-1} , and using 64 scans. The spectra were assigned to the same baseline and
100 normalized on the C-O stretching vibration of the glucopyranose ring of cellulose at 1060 cm^{-1} .
101

102 *2.4 Solid-state ^{13}C CP-MAS NMR spectroscopy*

103 ^{13}C magic angle spinning (MAS) NMR measurements were performed on a 500 MHz
104 Bruker Avance II NMR spectrometer WB (Wissembourg, France), working at frequency of
105 125.8 MHz (4 mm dual CPMAS (1H/BB)). Samples were placed into 4 mm Zirconia rotors
106 (Cortecnet, Paris) of various volumes (70 to $90\text{ }\mu\text{L}$), closed with a Kel-F cap, then spun at the
107 magic angle frequency set to 10 kHz . All spectra were recorded using a cross polarization,
108 CP, pulse sequence with a two-pulse phase modulation (TPPM) proton decoupling and with a
109 recycling delay of 5 s at 298 K. An average of 2000 scans was collected for each spectrum
110 and a Lorentzian filtering function of 3 Hz was applied. ^{13}C chemical shifts are reported
111 relative to the glycine external reference, whose carbonyl group was set at 176.03 ppm .

112 *2.5 Elaboration of the Pickering emulsions*

113 CNCs grafted with alkyl linear chains varying in lengths (from C2 to C18) and surface
114 degree of substitution ($DS_{\text{surf}} = 0.04$ to 0.66) were dispersed at the required concentration in

115 the continuous phase, being water or hexadecane according to the emulsion type. Two ml of
116 the different Pickering emulsions were prepared, with a W/O ratio of 80/20 v/v for direct
117 emulsions and 20/80 v/v for inverse emulsions. The amount of CNCs was adjusted to reach a
118 concentration of 8 mg of modified CNC per ml of oil phase (i.e. 3.2 and 12.8 mg of CNCs for
119 the 80/20 and 20/80 ratios, respectively). For direct emulsions, 0.4 mL of hexadecane was
120 added to 1.6mL of aqueous suspension of modified CNCs containing 50 mM NaCl. For
121 inverse emulsions, the modified CNCs were dispersed in hexadecane and 0.4 mL of water
122 containing fluorescein and 50 mM NaCl was added. In both cases, the mixture was then
123 sonicated with an ultrasonic device equipped with a dipping titanium probe (QSonica Q700,
124 150 J by alternating 2 s sonication with a 1 s standby for a total of 60 s).

125 *2.6 Determination of the emulsion type using fluorescence microscopy*

126 The emulsions were systematically visualized by transmission optical microscopy with
127 fluorescence detection in order to determine the type of emulsion formed (direct or inverse).
128 A total of 5 μ L of the resulting Pickering emulsion was poured onto a slide with spacer and
129 observed with a BX51 Olympus microscope.

130 *2.7 Droplet size analyses with increasing concentrations of grafted CNCs*

131 The droplet diameters distributions were measured by laser light scattering using a
132 Horiba LA-960 particle size distribution analyzer (Kyoto, Japan) for the different modified
133 CNCs at concentrations ranging from 0.4 to 16 mg of CNCs per ml of dispersed phase in the
134 range 0.01–5000 μ m. An analysis model was used with refractive index of 1.43 and 1.33 for
135 hexadecane and water, respectively. Measurements were carried out on direct emulsions only.
136 Depending on the samples, 50 to 200 μ l of the emulsions were introduced into the cell in
137 order to reach a transmittance around 95–97%. The alignment was performed before each
138 measurement and data acquisition times were set as default. The measurements were
139 systematically carried out in triplicate. The diameter was expressed as surface mean diameter

140 D(3,2) (Sauter diameter). The surface coverage (C) was determined from the ratio of
141 theoretical maximum surface area susceptible to be covered by the particles S_p , and the total
142 surface displayed by the radius of the oil droplets S_d (Kalashnikova, Bizot, Cathala, &
143 Capron, 2011):

$$144 \quad C = \frac{S_p}{S_d} = \frac{m_p D}{6h\rho V_{oil}} \quad \text{Eq. 1}$$

145 where m_p is the mass of CNCs introduced per mL of the dispersed phase, D is the sauter
146 mean diameter of the droplets (also called $D_{3,2}$), h is the thickness defined by AFM, ρ is the
147 CNCs density (1.6 g/cm³) and V_{oil} is the volume of oil included in the emulsion after
148 centrifugation.

149 **3. Results and discussion**

150 *3.1 Chemical modification of the CNCs*

151 The cellulose nanocrystals (CNCs) used in this study were obtained by sulphuric acid
152 hydrolysis of wood pulp. They consist of negatively charged rod-like particles (Zeta potential
153 of -51 ± 6 mV) with dimensions of 110 ± 48 nm in length and 4.8 ± 1.1 nm in thickness as
154 determined by atomic force microscopy (Brand, Pecastaings, & Sèbe, 2017; Dhuiège,
155 Pecastaings, & Sèbe, 2019). The amount of hydroxyl groups at their surface was estimated at
156 a value of 3.1 ± 0.1 mmol.g⁻¹, which corresponds to 16.7 % of the total number of OH groups
157 found in the particle (Brand, Pecastaings, & Sèbe, 2017). From this material, amphiphilic
158 CNCs were produced by substituting some of the hydrophilic surface hydroxyl groups by
159 hydrophobic acyl moieties, through an acylation reaction based on the transesterification of
160 vinyl esters and catalyzed by potassium carbonate (Fig. 1). During the reaction, the released
161 vinyl alcohol tautomerizes quickly to acetaldehyde, shifting the equilibrium towards the
162 formation of the acylated product (Brand, Pecastaings, & Sèbe, 2017). The
163 hydrophilic/hydrophobic balance at the CNCs surface was then finely monitored by varying i)
164 the chain length of the grafted acyl moieties (from C2 to C18) and ii) the reaction time.

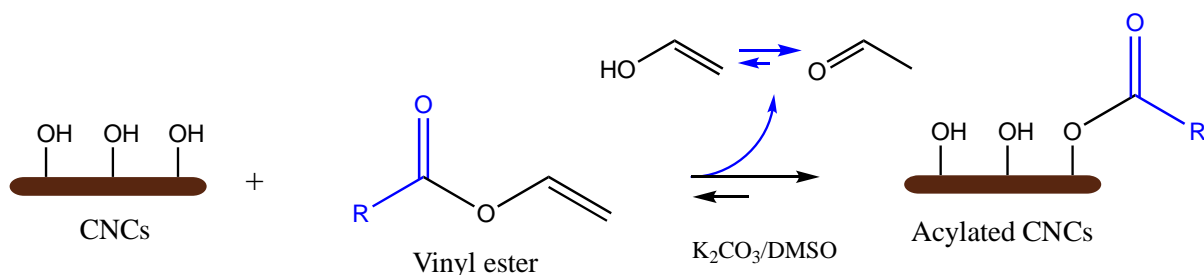
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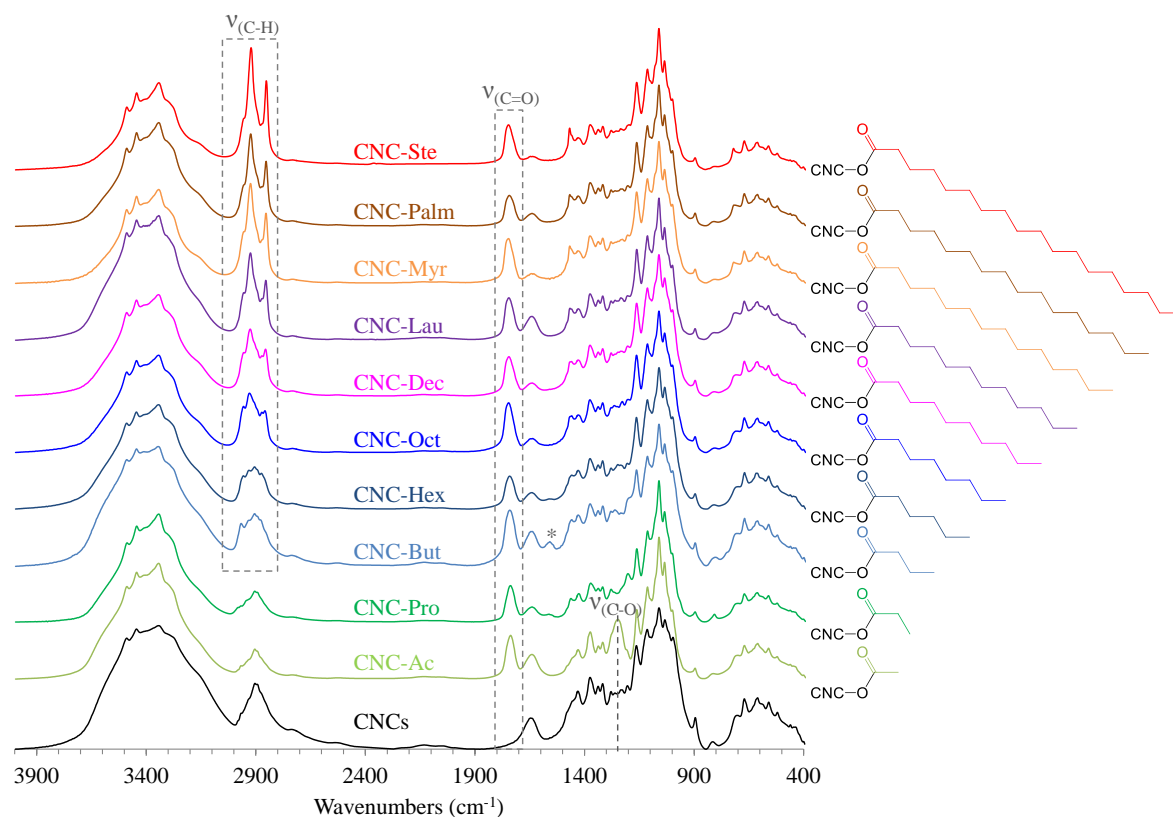


170

171 R = -CH₃ (CNC-Ac/C2), -CH₂CH₃ (CNC-Pro/C3), -CH₂CH₂CH₃ (CNC-But/C4), -
 172 CH₂[CH₂]₃-CH₃ (CNC-Hex/C6), -CH₂[CH₂]₅-CH₃ (CNC-Oct/C8), -CH₂[CH₂]₇-CH₃ (CNC-
 173 Dec/C10), -CH₂[CH₂]₉-CH₃ (CNC-Lau/C12), -CH₂[CH₂]₁₁-CH₃ (CNC-Myr/C14), -
 174 CH₂[CH₂]₁₃-CH₃ (CNC-Palm/C16), -CH₂[CH₂]₁₅-CH₃ (CNC-Ste/C18)

175 **Fig. 1.** General scheme for the surface acylation of the CNCs with vinyl esters of various
 176 chain lengths (in brackets: nomenclature of the sample/number of carbons in the
 177 corresponding grafted chain).

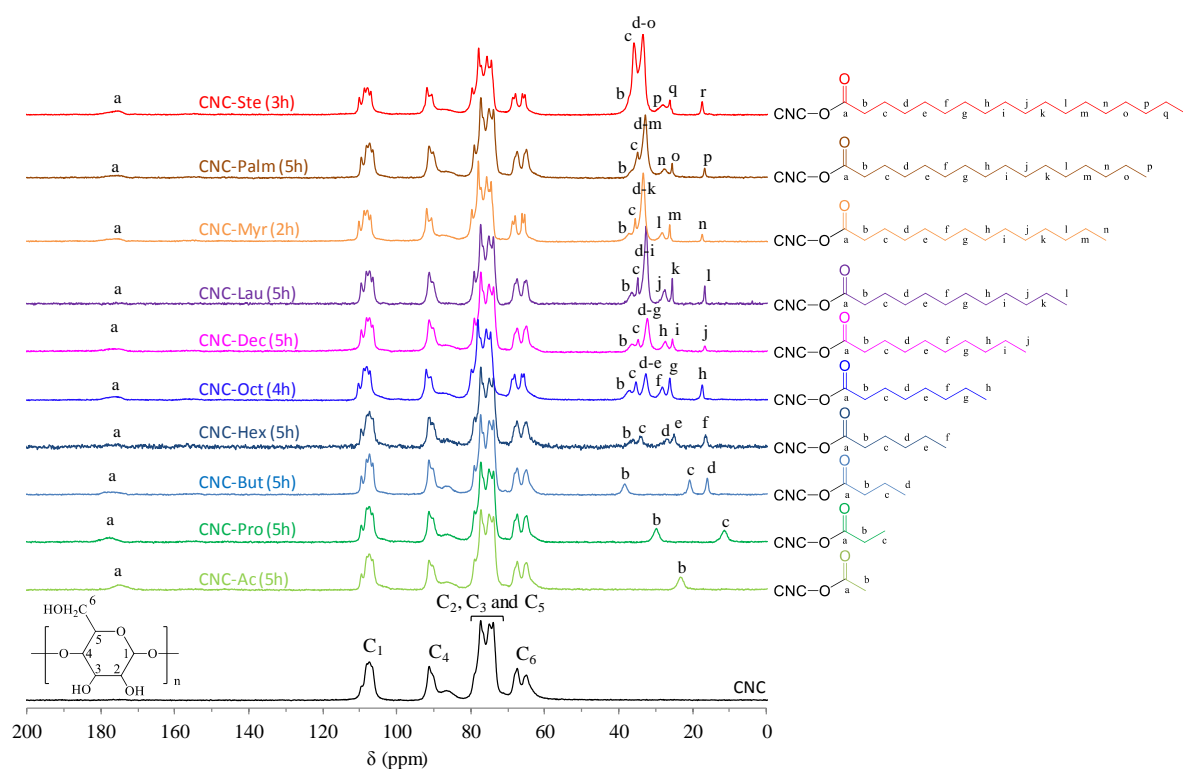
178 The efficiency of the reaction was confirmed by observing the characteristic vibrations
 179 and chemical shifts of the grafted acyl moieties in the FT-IR and ¹³C CP-MAS NMR spectra
 180 of the modified material (Fig. 2 and 3, respectively). In the FT-IR spectra, the stretching
 181 vibration of the carbonyl bond emerges at 1740 cm⁻¹ (ν_{C=O}), while the C-H stretchings of the
 182 CH₂ and -CH₃ groups are observed at 2925 and 2850 cm⁻¹. The C-O stretching of the ester
 183 bond is also observed at 1230 cm⁻¹ in the case of the acetylated sample (CNC-Ac).



184

185 **Fig. 2.** FTIR spectra of the unmodified and acylated CNCs after 5h of reaction. Traces of
 186 remaining potassium carbonate catalyst were sometimes observed (*).

187 In the ^{13}C CP-MAS NMR spectra (Fig. 3), the carbons of cellulose resonate at 110 ppm
 188 (C_1), 94 ppm (C_4 crystalline), 89 ppm (C_4 amorphous), 77-80 ppm (C_2 , C_3 and C_5), 70 ppm
 189 (C_6 crystalline) and 68 ppm (C_6 amorphous) (Attala, Gast, Sindorf, Bartuska, & Maciel,
 190 1980), while the grafted moieties can be seen at 175 ppm (signal of the carbonyl) and in the
 191 10-40 ppm region (signals of the methylene and methyl carbons). Whatever the sample, the
 192 NMR cellulose pattern was not modified by the acylation treatment and the signals
 193 corresponding to the crystalline domains retained their sharpness, which is consistent with a
 194 grafting limited to the outer surface of the nanoparticles.



195

196 **Fig. 3.** ^{13}C solid-state CP-MAS NMR spectra of the unmodified and acylated CNCs, with
 197 the assignment of the characteristic signals (reaction time in brackets).

198 The average degree of substitution (DS) per anhydroglucose unit (AGU) was deduced
 199 from the NMR spectra, by comparing the average integral per carbon for the grafted chain
 200 (between 0 and 40 ppm and at 180 ppm), with the integral of the C_1 carbon of cellulose used
 201 as reference:

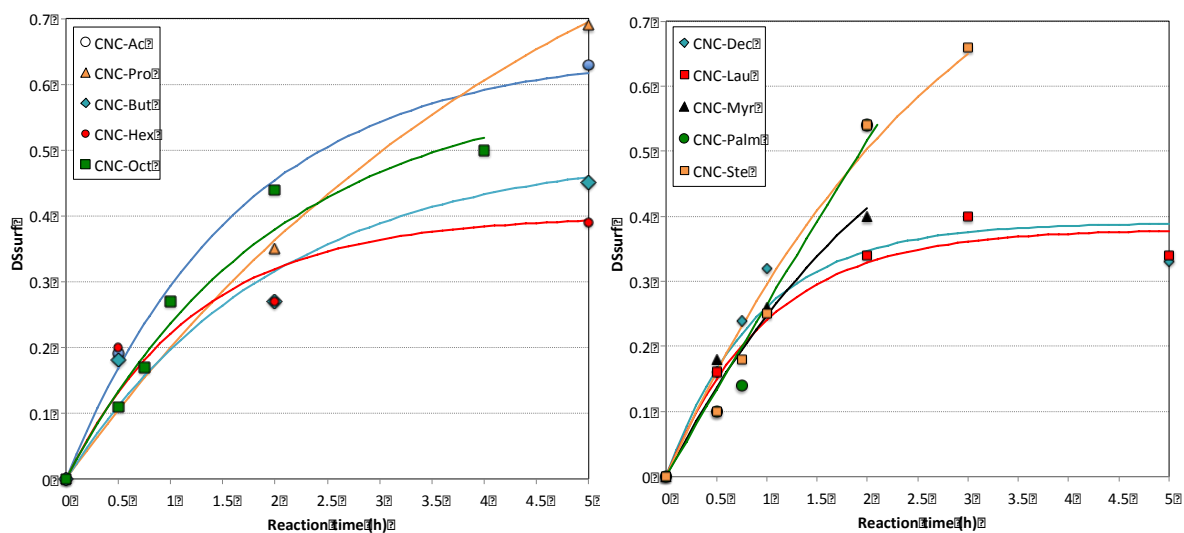
$$202 \quad \text{DS} = \frac{\text{Sum of the integrals for the grafted chain/number of carbons in the chain}}{\text{Integral of the C1 of cellulose}} \quad \text{Eq. 2}$$

203

204 This DS was later converted into a surface degree of substitution (DS_{Surf}), knowing that only
 205 16.7 % of the hydroxyl groups – i.e. 33.4 % of the total AGU – are located at the surface of
 206 the CNCs and hence accessible for a chemical reaction (Brand, Pecastaings, & Sèbe, 2017):

$$207 \quad \text{DS}_{\text{Surf}} = \text{DS}/0.334. \quad \text{Eq. 3}$$

208 As only 1.5 OH per AGU (in average) is truly accessible at the surface (Eyley, &
 209 Thielemans, 2014), the maximum value that the DS_{Surf} can reach is 1.5 (which is attained
 210 when all the surface OH groups are substituted). The extent of surface grafting over time was
 211 then quantified for each treatment, by plotting the DS_{Surf} as a function of reaction time
 212 (Fig. 4).



213
 214 **Fig. 4.** Evolution of the DS_{Surf} as a function of reaction time for the different acylated
 215 samples.

216 The reaction kinetics were quite similar for all treatments during the first hour ($DS_{Surf} =$
 217 0.2-0.3 after 1 h), but discrepancies were increasingly noted as the reactions further
 218 proceeded. In most cases, a DS_{Surf} of 0.4-0.5 was reached after 2 to 5 h of reaction, but a
 219 number of samples displayed a higher modification rate, namely CNC-Ac, CNC-Pro, CNC-
 220 Palm and CNC-Ste ($DS_{Surf} = 0.5$ to 0.7). The higher reactivity of the corresponding vinyl
 221 esters has not been explained at this stage of the study (the reactivity is obviously not simply
 222 related to the bulkiness of the reactant). The O_3 hydroxyl being involved in the strong O_3-
 223 $H \cdots O_5$ intrachain hydrogen bond (Eyley, & Thielemans, 2014), the reactivity of the hydroxyl
 224 groups at the cellulose surface should decrease in the order $O_6 \geq O_2 > O_3$, as was observed in
 225 the case of the heterogeneous acetylation of cellulose (Goodlett, Dougherty, & Patton, 1971).

226 Due to bulkiness of most vinyl esters, it is also likely that only the O₆ hydroxyl is modified
227 when the DS_{surf} is below 0.5 (i.e. when less than one third of the surface OH groups are
228 modified). Above this value, some O₂ hydroxyls should start to be acylated. In any case, the
229 different treatments performed here allowed obtaining a wide variety of amphiphilic CNCs
230 grafted with chains of various lengths (from C2 to C18) and various surface degree of
231 substitution (DS_{surf} = 0.1 to 0.7), which were used as model Pickering particles in subsequent
232 investigations.

233 *3.2 Impact of grafting on direct O/W emulsions*

234 In a first set of experiments, various emulsions were prepared by sonicating the CNCs
235 aqueous suspensions with hexadecane, at a W/O volume ratio of 80/20, and observed after
236 24h. The type of emulsion (O/W or W/O) was inferred by pouring a drop of emulsion in a
237 volume of either hexadecane or water (Fig S1 in Supplementary Material). In these
238 conditions, stable O/W emulsions were systematically obtained with the particles grafted with
239 short acyl chains (C2 to C6), regardless of the DS_{surf}. For chains with more than 8 carbons,
240 dense and resistant O/W emulsions could be produced only when the DS_{surf} was ≤ 0.15 , the
241 emulsions being unstable at higher DS_{surf} (Fig. 5). This behavior is directly linked to the high
242 hydrophobicity of the acyl groups of 8 carbons or more, which confer an excessive
243 hydrophobicity to the particles when DS_{surf} > 0.15, leading to unstable direct O/W emulsions
244 (Binks, & Lumsdon, 2000).

245

	10'	15'	30'	45'	1H	2H
C8	$DS_{surf} = 0.04$	$DS_{surf} = 0.07$	$DS_{surf} = 0.13$	$DS_{surf} = 0.19$	$DS_{surf} = 0.24$	$DS_{surf} = 0.38$
C10	$DS_{surf} = 0.06$	$DS_{surf} = 0.09$	$DS_{surf} = 0.16$	$DS_{surf} = 0.22$	$DS_{surf} = 0.26$	$DS_{surf} = 0.35$
C12	$DS_{surf} = 0.05$	$DS_{surf} = 0.08$	$DS_{surf} = 0.15$	$DS_{surf} = 0.20$	$DS_{surf} = 0.24$	$DS_{surf} = 0.33$
C14	$DS_{surf} = 0.04$	$DS_{surf} = 0.07$	$DS_{surf} = 0.14$	$DS_{surf} = 0.19$	$DS_{surf} = 0.25$	$DS_{surf} = 0.41$
C16	$DS_{surf} = 0.04$	$DS_{surf} = 0.07$	$DS_{surf} = 0.13$	$DS_{surf} = 0.20$	$DS_{surf} = 0.26$	$DS_{surf} = 0.52$
C18	$DS_{surf} = 0.05$	$DS_{surf} = 0.08$	$DS_{surf} = 0.16$	$DS_{surf} = 0.23$	$DS_{surf} = 0.30$	$DS_{surf} = 0.50$

246

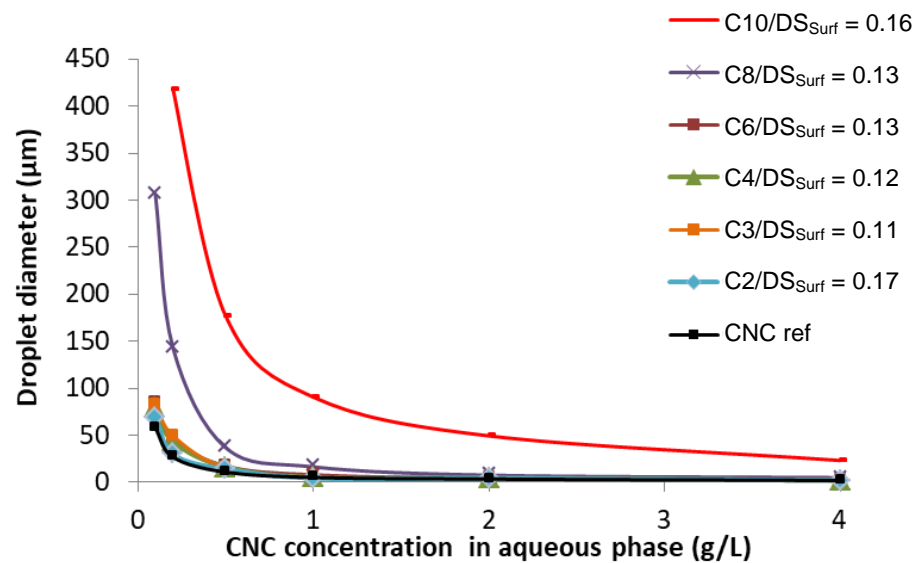
247 **Fig. 5.** Pictures of the hexadecane-in-water emulsions stabilized by the CNCs modified with
 248 long acyl chains (C8 to C18) and various DS_{surf} (obtained after 10', 15', 30', 45', 1h and 2h of
 249 reaction), observed after 24h of storage at room temperature (W/O ratio = 80/20 v/v). The
 250 DS_{surf} estimated from the trend curves in Fig. 4 are also reported.

251 The CNCs concentration dependence on the average drop diameter of the direct O/W
 252 emulsions was determined for the different CNC samples. When the concentration of native
 253 CNCs was increased from 0.1 to 4 g/L in the aqueous phase, the droplet diameter of the
 254 emulsion decreased until reaching a plateau at $3\mu\text{m}$ (Fig. 6). This behavior is in accordance
 255 with the so-called limited coalescence process (Arditty, Whitby, Binks, & Leam-Calderon,
 256 2003). After sonication, the uncovered droplets merge; this coalescence is stopped when the

257 minimum drop coverage required for stability is reached. The drop diameter depends then
258 directly on the amount of particle available to stabilize the interface. The second part of the
259 curve presents a plateau. It occurs when the drop diameter cannot decrease anymore; the
260 system will evolve at constant interface area. In many systems, the particles are released in
261 the continuous phase but rods can move at the interface and orientate into parallel bundles
262 leading to densification of the CNC organization (Cherhal, Cousin, & Capron, 2016). The
263 same profile was observed in Fig. 6 for CNCs modified with short acyl chains (from C2 to
264 C6), but differences were noted with the CNCs grafted with longer hydrophobic chains (\geq
265 C8). For these samples, a higher droplet diameter is observed at a given particles
266 concentration and degree of substitution, as well as a shift in the concentration needed to
267 reach the plateau at $3\mu\text{m}$ (Fig. 6). The limited coalescence domain is controlled by the
268 irreversible adsorption of particles at the interface. When the surface is totally covered, a
269 plateau in drop diameter is observed. The limit concentration between these two domains was
270 evaluated at 0.5 g/L for unmodified CNCs, 2 g/L for the CNCs grafted with C2 to C8, and
271 more than 4 g/L for those grafted with C10. Hence, there is a clear impact of chain length on
272 the stabilization process for direct O/W emulsions, which could result from an increasing
273 aggregation of the CNCs at the droplets surface, through hydrophobic interactions, leading to
274 a decrease of the total surface available for the stabilization of the O/W interface.

275 A similar result was obtained when the hydrophobicity at the surface of the particles was
276 progressively increased with a single acyl group, through the incrementation of the DS_{Surf}
277 (Fig. 7a). To further evaluate the organization of the particles at the interface, the coverage
278 percentage (C) for the various samples was estimated with Eq. 1, from the slope of the
279 reciprocal surface-average diameter ($1/D$) plotted as a function of CNCs concentration (for
280 direct emulsions, and expressed in mg per ml of oil phase) (Fig. 7b). The coverage ratio
281 decreases progressively from a fully covered surface with a value of 107 %, to a slightly

282 covered surface with a value of 27% when the DS_{surf} increased from 0 to 0.19 respectively,
283 confirming the enhanced aggregation state of the CNCs at higher modification rate. A similar
284 aggregation has been reported in the literature after reduction of the electrostatic repulsions
285 between native CNCs, through desulfatation of their surface (Cherhal, Cousin, & Capron,
286 2016), leading to a more porous and heterogeneous droplet surface.

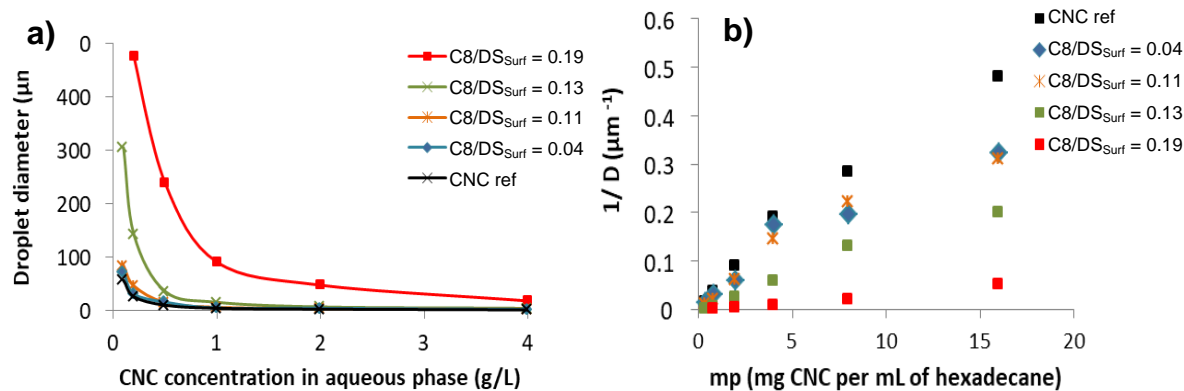


287
288 **Fig. 6.** Evolution of the direct emulsion droplet diameter with increasing particles
289 concentration for CNCs grafted with acyl chains of C2 to C10, with a DS_{surf} in the same order
290 of magnitude (the DS_{surf} was estimated from the trend curves in Fig. 4).

291

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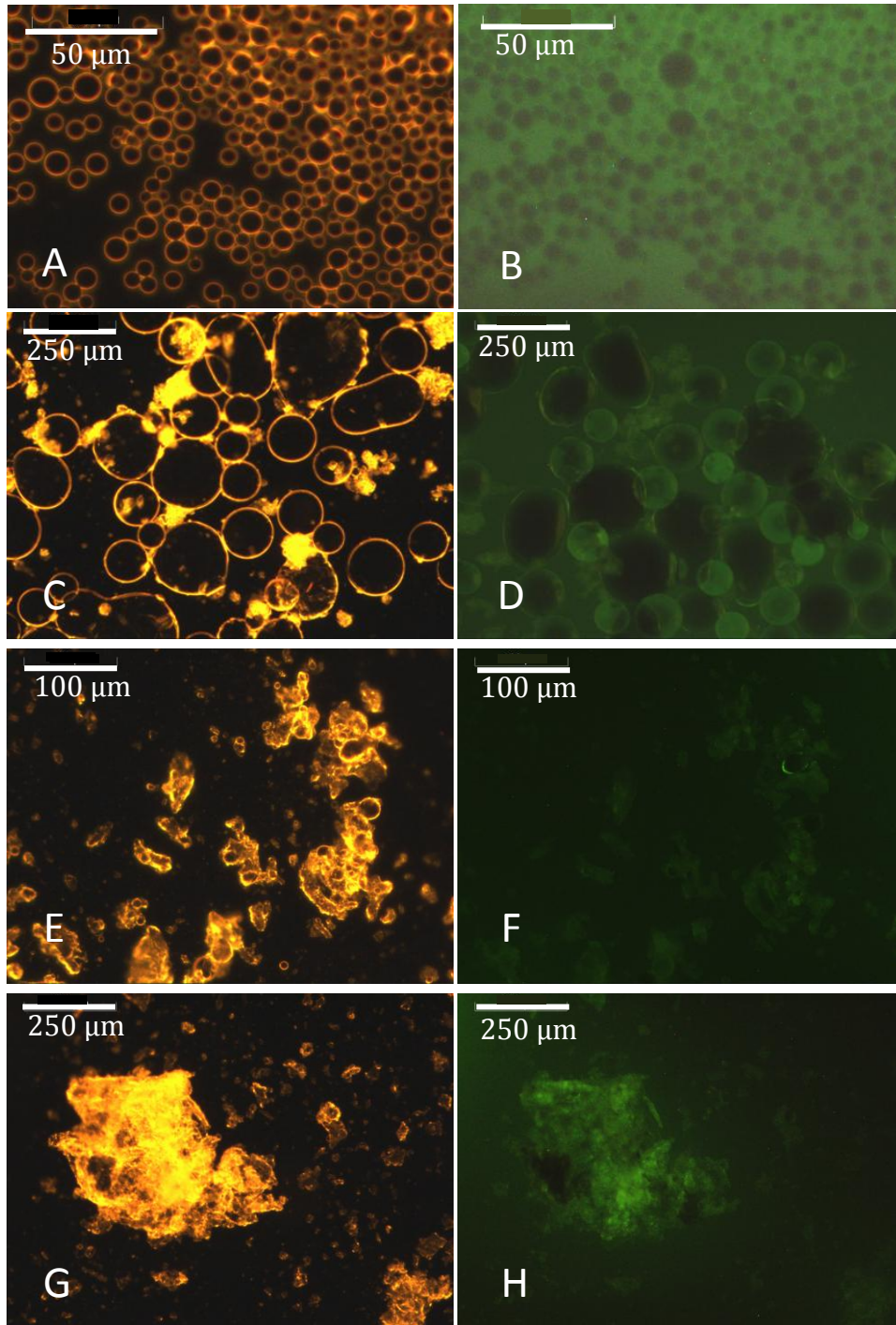
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294
 295 **Fig. 7.** **a)** Evolution of the direct emulsion droplet diameter with increasing particles
 296 concentration for the CNCs grafted with C8 acyl chains of various DS_{surf} (the DS_{surf} was
 297 estimated from the trend curves in Fig. 4). **b)** Inverse D(3,2) diameter plotted as a function of
 298 particles concentration (expressed in mass of CNCs per mL of hexadecane).

299 All the O/W emulsions were further confirmed by staining the water continuous phase
 300 with fluorescein (appearing in green) and visualizing the droplets with a fluorescence
 301 microscope. Some typical results are exemplified in Fig. 8. The CNCs grafted with short acyl
 302 chains (C2 to C6, regardless of the DS_{surf}), or long chains and low DS_{surf} (C8 to C18 and
 303 DS_{surf} ≤ 0.15), led to O/W emulsions with small oil droplets (about 3 μm) and low size
 304 distributions in the images (Fig. 8A-B). The oil droplets that are creaming can be seen in
 305 black in contact with the slide in Fig. 8B, while the continuous phase appears clearly in green.

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311 **Fig. 8.** Optical (A, C, E, G) and fluorescence (B, D, F, H) microscopy images of a typical
 312 (A-B) direct O/W emulsion (here: C8-graft with $DS_{\text{surf}} = 0.13$), (C-D) intermediate state
 313 where oil droplets co-exist with aggregated water droplets (here: C8-graft with $DS_{\text{surf}} = 0.24$),
 314 and (E-H) inverse W/O emulsion (here: C8-graft with $DS_{\text{surf}} = 0.38$).

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316 3.3 Inverse W/O emulsions

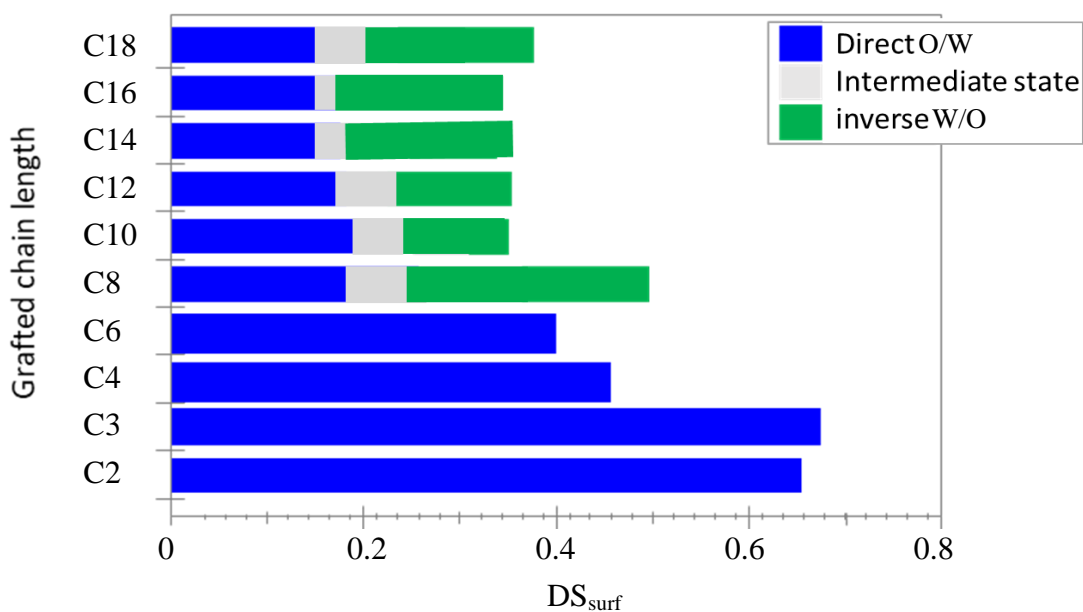
317 In a second set of experiments, emulsions were prepared by dispersing the hydrophobized
318 CNCs in the oil phase, then sonicating the suspension with a lower volume of water (W/O =
319 20/80 v/v), with the objective to produce inverse emulsions. Here again, the type of emulsion
320 was inferred by observing what happens when a drop of emulsion was added to a volume of
321 either hexadecane or water (Fig. S1 in Supplementary Material). In these conditions, only the
322 particles grafted with acyl chains of 8 carbons or more produced inverse W/O emulsions, and
323 only when the particles surface was sufficiently modified (i.e. when DS_{surf} was above 0.2-0.3,
324 depending on the acyl chain). The formation of W/O emulsions with the longer acyl chains
325 was further confirmed by fluorescence microscopy, the water encapsulated in the oil phase
326 appearing in the form of various irregular structures that may correspond to coalesced water
327 droplets (Fig. 8E-G and Fig. S2 in Supplementary Material). It has been indeed shown in a
328 comprehensive study by Simovic & Prestidge 2004, that for hydrophobic nanoparticles coated
329 droplets, the limited coalescence process can result in a flocculated network composed of
330 coalesced droplets of irregular shapes. At intermediate DS_{surf} , the microscopy images revealed
331 an intermediate state, where creaming oil droplets seem to co-exist with aggregated water
332 droplets (Fig. 8C-D). These microscopic observations are quite puzzling, but could be related
333 to the co-existence of CNCs of low and high acylation levels when the reaction time is
334 intermediate; the initial cellulosic substrate is indeed quite variable and the calculated DS_{surf}
335 represents only an average estimate. Besides, the oil droplets observed in this intermediate
336 state are larger than those of the direct emulsions, confirming that less low-acylated particles
337 are available to stabilize this interface, some of the particles (the more acylated ones) being
338 probably consumed by the interface of the water droplets seen in the microscopic images.

339 The different types of emulsions obtained in our experiments are summarized in Fig. 9, as
340 a function of chain length (C2 to C18) and surface degree of substitution ($DS_{\text{surf}} = 0.04$ to

341 0.69). These results show that, with the DS_{surf} obtained in our experimental conditions (DS_{surf}
342 $\leq 0,7$), it is never possible to produce inverse emulsions with short alkyl chains (C2 to C6). In
343 the better case, less than 50% of the surface OH groups are substituted (the theoretical
344 maximum DS_{surf} being 1.5), which is too low to impart a sufficient hydrophobicity to the
345 CNCs surface and stabilize W/O emulsions with such short lengths. Distinctively, all the
346 particles grafted with more than 8 carbons allow the stabilization of both direct and inverse
347 emulsions, depending on the level of grafting. If O/W emulsions necessitate low DS_{surf} , the
348 stabilization of W/O emulsions is possible only when the CNCs surface is sufficiently
349 modified by the long chains. Beyond 8 carbons, inverse emulsions are indeed systematically
350 produced if $DS_{surf} > 0.25$, i.e. when about one third of the surface OH groups are substituted.
351 However, long chains will tend to entangle via hydrophobic interactions, leading to large
352 particles assemblies and low surface coverage of the droplets at the interface; more material is
353 consequently required for an efficient surface stabilization in that case. It can be noted that
354 similar results were obtained when the hexadecane oil was replaced by toluene (results not
355 shown).

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359 **Fig. 9.** Type of emulsion obtained in our experimental conditions as a function of DS_{surf} and
 360 number of carbons in the grafted chain. Direct and inverse emulsions are obtained with W/O
 361 = 80/20 and 20/80 v/v, respectively. The intermediate state corresponds to a situation where
 362 both oil and water droplets are present in the medium according to the microscopic
 363 observations.

364 4. Conclusions

365 This work investigates the potential of surface acylation as a tool to monitor the
366 properties of Pickering emulsions stabilized by cellulose nanocrystals (CNCs), through the
367 control of the grafted chain length and grafting level. Amphiphilic CNCs grafted with acyl
368 groups of various chain lengths and surface degree of substitution (DS_{surf}) were produced,
369 through an acylation reaction based on the transesterification of vinyl esters. The grafting was
370 confirmed by FT-IR and ^{13}C CP-MAS NMR spectroscopy, and quantified by the calculation
371 of the surface degree of substitution (DS_{surf}) obtained from the NMR spectra. Whatever the
372 DS_{surf} , CNCs grafted with linear acyl chains of 2 to 6 carbons led to the exclusive formation
373 of direct O/W emulsions with hexadecane. When the linear chain contained 8 carbons or
374 more, both O/W and W/O emulsions could be obtained at low and high DS_{surf} , respectively.
375 The inverse W/O emulsions could be stabilized only when at least one third of the surface OH
376 groups were substituted. At intermediate DS_{surf} , the microscopic investigations revealed the
377 existence of an intermediate situation where both oil and water droplets seem to co-exist,
378 which could be related to the variability of the acylation process (co-existence of CNCs of
379 low and high acylation levels). The chain length and DS_{surf} had a clear impact on the
380 stabilization process for direct O/W emulsions, larger droplets being obtained for a given
381 particles concentration at higher chain length and DS_{surf} . This was assigned to an increasing
382 aggregation of the CNCs at the droplets surface, through hydrophobic interactions, leading to
383 a decrease of the total surface available for the stabilization of the O/W interface. As a
384 consequence, the surface coverage of the emulsion droplets is significantly varied, which
385 should have an impact on the permeability of the Pickering capsules in delivery applications.
386 In any case, we were able to monitor the type of emulsion formed, droplet size and surface
387 coverage at the oil/water interface, by adjusting the length of the grafted chain, DS_{surf} , and
388 particles concentration.

389

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395 **Supplementary material**

396 Supplementary material related to this article can be found in the online version.

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