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1           **Pickering emulsions stabilised by hydrophobically**  
2           **modified cellulose nanocrystals: responsiveness to pH**  
3           **and ionic strength**

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26 **Abstract**

27 The aims of this study were to hydrophobically modify cellulose nanocrystals  
28 (CNCs), investigate the ability of such modified CNCs (MCNCs) to stabilise Pickering  
29 oil-in-water (O/W) emulsions and understand their stability at different pHs (2.0–7.0)  
30 and ionic strengths (0–150 mM NaCl). Structural changes that resulted from  
31 esterifying CNCs with octenyl succinic anhydride (OSA) were determined using  
32 Fourier transform infrared (FTIR) spectroscopy, X-ray diffractometry (XRD),  
33 transmission electron microscopy (TEM) and wettability analysis. The stability of the  
34 Pickering O/W emulsions (20 wt% oil, 0.05–1.00 wt% MCNCs) was assessed using  
35 droplet sizing, microscopy,  $\zeta$ -potential, apparent viscosity and oscillatory rheological  
36 measurements. FTIR spectroscopy confirmed a decrease in the intensity of the –OH-  
37 associated band because of reaction of the hydroxyl group with OSA. XRD indicated  
38 a lower (11.5%) crystallinity index in MCNCs. TEM revealed that there was no  
39 change in morphology of the needle-shaped CNCs upon OSA modification  
40 (length/diameter = 40–100 nm/2–4 nm). Hydrophobic modification of CNCs with OSA  
41 was evidenced by an increase in static water contact angle from 56° (untreated  
42 CNCs) to 80.2° (MCNCs) which allowed the MCNCs to be partially wetted by both  
43 the phases and stabilise O/W emulsions. The Pickering emulsions showed droplet  
44 flocculation at pH < 4.0 (without addition of NaCl) or ionic strength  $\geq$  20 mM NaCl  
45 (pH 7.0), with a predominant elastic gel-like behaviour observed at  $\geq$  20 mM NaCl.  
46 Resistance of MCNC-based Pickering emulsions to coalescence and  
47 responsiveness to flocculation at bio-relevant pHs and ionic strengths show promise  
48 in the design of delivery vehicles.

49

50 **Keywords:** Cellulose nanocrystal; Octenyl succinic anhydride; Pickering emulsion;  
51 Particle-stabilised emulsion; Electrostatic interaction

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## 58 **1. Introduction**

59 Pickering emulsions, i.e. emulsions stabilised by solid particles, have gained  
60 significant research attention from food colloid scientists because of their combined  
61 advantages of high resistance to coalescence and the ability to delay lipid digestion  
62 (Dickinson, 2012; Sarkar, Zhang, Holmes, & Ettelaie, 2019). Pickering emulsions can  
63 be either oil-in-water (O/W) or water-in-oil (W/O) depending on the preferential  
64 wettability of the stabilising particles (Chevalier & Bolzinger, 2013). Hydrophilic  
65 particles favour the formation of O/W emulsions, whereas hydrophobic particles are  
66 generally suitable for W/O emulsions (Zembyla, Murray, & Sarkar, 2018). Pickering  
67 O/W emulsions using inorganic particles have been studied extensively, but the use  
68 of biocompatible particles, such as protein microgels (Destribats, Rouvet, Gehin-  
69 Delval, Schmitt, & Binks, 2014; Hu et al., 2016; Sarkar et al., 2016), modified starch  
70 and non-starch polysaccharide particles (Li et al., 2018; Merefati, Matos, Wiege,  
71 Haase, & Rayner, 2018; Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011;  
72 Yusoff & Murray, 2011), protein–protein particle composites (Liu, Huang, Chen,  
73 Deng, & Yang, 2019), protein–polysaccharide particle composites (Doost et al.,  
74 2019; Sarkar et al., 2018a) and polysaccharide–polysaccharide particle composites  
75 (Li et al., 2019), for generating particle-stabilised interfaces is relatively recent. Such  
76 particles have been studied mainly to stabilise Pickering emulsions with the ultimate  
77 aim being to alter the kinetics of lipid digestion.

78 For the design of small-sized (1–10  $\mu\text{m}$ ) oil droplets, as solid stabilisers must  
79 be in the nanometric size range, modified starch granules might not be suitable  
80 Pickering stabilisers because they are generally several microns in size unless they  
81 have been modified by physical approaches (Yusoff & Murray, 2011). Although  
82 protein and inulin particles have been reported to have nanometric sizes, emulsions  
83 stabilised by protein/inulin composites or protein particles as the sole stabiliser had  
84 droplet sizes ( $d_{43}$ ) of up to 24.7  $\mu\text{m}$  (Sarkar, Li, Cray, & Boxall, 2018b) and larger  
85 than 100  $\mu\text{m}$  in the case of zein particles (de Folter, van Ruijven, & Velikov, 2012). In  
86 addition, as protein-based particle-stabilised emulsions are generally sensitive to  
87 coalescence under physiological conditions because of their susceptibility to  
88 proteolytic enzymes (Sarkar et al., 2018a), there has recently been a demand for  
89 particles that are resistant to human gastrointestinal enzymes as potential Pickering  
90 stabilisers.

91 Interestingly, needle-shaped cellulose nanocrystals (CNCs) with a  
92 characteristic length of around 100–200 nm and a diameter of 8–86 nm, depending  
93 on their source (Chen et al., 2018; Lee et al., 2011; Yan et al., 2017), are human-  
94 enzyme-resistant particles that have attracted recent research attention for the  
95 production of stable Pickering emulsions. However, as CNCs are known to be highly  
96 hydrophilic (Cherhal, Cousin, & Capron, 2016), their partial wettability by a  
97 hydrophobic phase can be expected to be poor, which may limit their direct use as  
98 Pickering stabilisers. Hence, the first method to improve the wettability of these  
99 CNCs was to combine them with surfactants. For example, CNCs were blended with  
100 surfactants and these particle–surfactant mixtures were used to create the particle-  
101 laden interface (Hu, Ballinger, Pelton, & Cranston, 2015). Another approach was to  
102 use protein as a primary layer and then to add CNCs to the system as a second  
103 layer to form a composite protein–CNC interface (Sarkar et al., 2018b; Sarkar,  
104 Zhang, Murray, Russell, & Boxal, 2017); however, whether in such a system, a true  
105 particle-stabilization mechanism was there can be argued. In both approaches, as  
106 the emulsions were probably stabilised by surfactants and not by the solid CNCs, the  
107 high desorption energies associated with true Pickering stabilisers might not be  
108 achieved in these systems.

109 An alternative approach is hydrophobic modification of the CNCs with various  
110 substances, such as succinic anhydride (Liu, Sun, Zhang, Ren, & Geng, 2006),  
111 octenyl succinic anhydride (OSA) (Chen et al., 2018), phenyltrimethylammonium  
112 chloride (Gong, Wang, & Chen, 2017) and organic acids (hexanoic acid and  
113 dodecanoic acid) (Lee et al., 2011). Such modified CNCs (MCNCs) can be used as  
114 the sole stabiliser without the need for any surfactant or protein. Of all the chemicals  
115 mentioned above, OSA, which has been used for decades in the food industry for  
116 the modification of starch (Nilsson & Bergenståhl, 2007; da Silva et al., 2013), seems  
117 to be the most straightforward. Only one study has reported on the modification of  
118 CNCs using OSA to produce high internal phase emulsion gels (Chen et al., 2018).  
119 To our knowledge, there is no published literature on the creation of Pickering O/W  
120 emulsions using OSA-modified CNCs. In addition, there is no study to date that has  
121 systematically investigated the behaviour of OSA-modified CNC-stabilised Pickering  
122 emulsions when subjected to environmental stresses, such as pH and electrolytes.  
123 Responses of the emulsions to such environmental stresses are crucial to understand

124 the fate of these emulsions during physiological transit when they are used for  
125 designing delivery systems.

126 Therefore, the aim of this study was to systematically characterise the  
127 modification of CNCs using OSA and investigate the physicochemical and  
128 microstructural behaviour of the O/W emulsions stabilised by OSA-modified CNCs  
129 as a function pH and ionic strengths. We used a combination of light scattering,  
130 confocal laser scanning microscopy, electron microscopy, electrophoretic mobility  
131 measurements, bulk rheology and spectroscopic techniques to examine the ability of  
132 these modified particles to create stable small-sized Pickering emulsion droplets and  
133 to study the stability of the droplets under various environmental conditions. To the  
134 best of our knowledge, this is the first work on the characterisation of MCNC-based  
135 Pickering emulsions and the responsiveness of these emulsions to pH and ionic  
136 strength. As this is the first study of a series of work being conducted by the authors  
137 on delivery of short chain fatty acids, a mixture of sunflower oil (SFO) with  
138 tripropionin [glyceryl tripropionate (TPP), a source of propionic acid] and tributyrin  
139 [glyceryl tributyrate (TBT), a source of butyric acid] was used as the dispersed phase  
140 for the production of the Pickering O/W emulsions.

141

## 142 **2. Materials and methods**

### 143 **2.1. Materials**

144 CNCs (94-96% sulphated CNCs) derived from sulphuric acid treatment of bleached  
145 Kraft pulp were purchased from [CelluForce™, Montreal, Canada](#). The detailed  
146 chemical composition of CNCs can be obtained from a previous study ([Reid et al.,  
147 2017](#)). The CNCs used in this study was intended for research purposes and not for  
148 consumption. SFO was purchased from a local supermarket (Morrisons, [Leeds, UK](#)).  
149 Food-grade tripropionin ( $\geq 97.1\%$  TPP), tributyrin ( $\geq 97.1\%$  TBT), sodium azide and  
150 OSA were purchased from [Sigma-Aldrich Company Ltd, Dorset, UK](#). All other  
151 chemicals were of analytical grade and were purchased from [Sigma-Aldrich  
152 Company Ltd, Dorset, UK](#). Milli-Q water (ionic purity of 18.2 M $\Omega$ .cm at 25 °C) purified  
153 by a [Milli-Q apparatus \(Millipore Corp., USA\)](#) was used as the solvent for all  
154 experiments. Sodium azide (0.02 wt%) was used as a bactericide to prevent  
155 microbial growth during refrigerated storage (at 4 °C) of the samples.

156

## 157 **2.2. Hydrophobic modification of CNCs**

158 Hydrophobic modification of CNCs was conducted according to a method that has  
159 previously been used for inulin ([Han, Ratcliffe, & Williams, 2015](#)) with some  
160 modifications. All reactions were performed in a 500 mL round-bottomed flask at 25  
161 °C. The CNC dispersion (3.0 wt% in water) was mixed with OSA at a ratio of 1:0.15  
162 (w/w). The pH was adjusted to pH  $8.30 \pm 0.1$  using 0.5 N NaOH. During the  
163 esterification reaction of hydroxyl groups on the CNC backbone with OSA, the pH  
164 was maintained by the continuous addition of 0.5 N NaOH using a pH-stat ([TIM856,](#)  
165 [Radiometer Analytical, Hach Company, Loveland, CO, USA](#)). The reaction was  
166 carried out until no further NaOH was needed to neutralise the acidic products to  
167 ensure that all the OSA had been consumed; typically, the time required was around  
168 7.0 h. Once the reaction was complete, the resultant product was neutralised to pH  
169 7.0 with 1.0 N HCl and then lyophilised, yielding a white powder. The powder was  
170 purified by Soxhlet extraction for 12 h using ethanol to remove any leftover OSA from  
171 the powder. Finally, it was air dried in an oven at 40 °C overnight to remove the  
172 ethanol. This powder is referred to as modified cellulose nanocrystals (MCNCs) and  
173 was used to create Pickering O/W emulsions.

174

## 175 **2.3. Structural analysis of MCNCs**

### 176 *2.3.1. Fourier transform infrared (FTIR) spectroscopy*

177 Before measurement, the MCNCs were ground sufficiently with a mortar and pestle;  
178 all samples were then pressed into pellets. The measurements were carried out  
179 using a Bruker ATR-FTIR spectrometer ([Bruker Optics GmbH, Ettlingen, Germany](#))  
180 in the mid-IR region ( $400\text{--}4000\text{ cm}^{-1}$ ), with a resolution of  $4\text{ cm}^{-1}$  for at least 64  
181 scans. The FTIR spectra of unmodified CNCs and MCNCs were collected as  
182 average values of 64 scans using [Mobility Series™](#) software before being exported  
183 to Origin 2016 Sr2 ([OriginLab Corp., Northampton, MA, USA](#)) for peak fitting.

184

### 185 *2.3.2. X-ray diffractometry (XRD)*

186 XRD profiles of unmodified CNCs and MCNCs were obtained using a [Bruker AXS](#)  
187 [D8 Advance diffractometer](#) with LINXEYE detector using Cu K $\alpha$  ( $\lambda = 0.154$  nm). The  
188 XRD analysis was performed at room temperature at 40 kV and 40 mA, with  $2\theta$   
189 ranging from 0 to 60°, at a scan rate of 0.02° min<sup>-1</sup>. The crystallinity indexes (CIs) of  
190 the CNCs and MCNCs were calculated using the Segal method, as described  
191 previously ([Lee et al., 2011](#)):

192

$$193 \quad CI = \left( \frac{I_{002} - I_{AM}}{I_{002}} \times 100\% \right) \quad (1)$$

194

195 where  $I_{002}$  is the peak intensity of plane 002 and  $I_{AM}$  is the minimum intensity  
196 between planes 002 and  $10\bar{1}$  in the XRD profiles.

197

### 198 *2.3.3. Wettability*

199 Static water contact angles of unmodified CNCs and MCNCs were determined at 25  
200 °C with the sessile drop method using an OCA25 drop-shape tensiometer  
201 ([DataPhysics Instruments, Filderstadt, Germany](#)) fitted with a microsyringe and a  
202 high speed IDS camera. Before measurement, both CNCs and MCNCs were  
203 pressed, under a weight of 6 tonnes for 30 s, between the plates of a hydraulic  
204 bench press (Clarke, Kempston, UK) into discs with diameters of 15 mm and  
205 thicknesses of approximately 2 mm, according to a previous method ([Zembyla et al.,](#)  
206 [2018](#)). A straight needle (0.52 mm outer diameter and 0.26 mm internal diameter)  
207 was used to produce 5.0  $\mu$ L water droplets that formed sessile drops on the discs  
208 made from unmodified CNCs or MCNCs. The water droplet contour was recorded  
209 using a video camera and was immediately fitted using the [SCA202 V5.0.15](#)  
210 software. Static water contact angles ( $\theta_w$ ) between the CNC or MCNC disc and the  
211 water were then measured. All measurements were done in triplicate and were  
212 reported as the mean and standard deviation.

213

### 214 *2.3.4. Transmission electron microscopy (TEM)*

215 TEM was used to observe the structural changes in the CNCs (if any) after  
216 modification with OSA. Both CNC and MCNC samples (10  $\mu\text{L}$ ) were stabilised with  
217 2.5% (v/v) glutaraldehyde, fixed in 0.1% (w/v) osmium tetroxide and then embedded  
218 in araldite. Ultra-thin sections (80–100 nm) were then placed on 3.05 mm grids and  
219 stained with 8% (v/v) uranyl acetate and lead citrate. The imaging was carried out  
220 using a [CM10 TEM microscope \(Philips, Guildford, UK\)](#).

221

#### 222 **2.4. Preparation of Pickering O/W emulsions**

223 The MCNCs were used as Pickering stabilisers at various concentrations to create  
224 O/W emulsions. The oil phase (TPP–TBT–SFO mixture with a weight ratio of 1:1:2)  
225 was pre-homogenised at an oil:aqueous phase ratio of 1:4 (w/w) using a high speed  
226 blender ([D500 series, Biolab Ltd, Germany](#)) at 10,000  $\text{rev min}^{-1}$  for 3 min. The  
227 emulsions were prepared using various concentrations of MCNCs, i.e. 0.05, 0.10,  
228 0.20, 0.50 and 1.0 wt% in the final emulsions, hereafter referred to as emulsions  
229 E0.05, E0.10, E0.20, E0.50 and E1.00 respectively. In the next step, the coarse  
230 emulsions were homogenised using a two-stage valve homogeniser ([Panda Plus,  
231 GEA Niro Soavi, Parma, Italy](#)) at pressures of 200/50 bar for three passes. The  
232 resulting emulsions were used for analysis of droplet size,  $\zeta$ -potential, microstructure  
233 and stability. Control emulsions were also prepared from unmodified CNCs using 1.0  
234 wt% CNCs.

235

#### 236 **2.5. Characterisation of Pickering O/W emulsion droplets**

237 The emulsions were characterised for droplet size,  $\zeta$ -potential and viscosity, and the  
238 microstructure was assessed using confocal laser scanning microscopy (CLSM).  
239 The samples were diluted to around 0.01% w/v for measurement of the droplet size  
240 and the  $\zeta$ -potential. Droplet size distributions of the emulsions were determined at 25  
241  $^{\circ}\text{C}$  by static light scattering using a Mastersizer ([3000S series, Malvern Instruments  
242 Ltd, Malvern, UK](#)). The relative refractive index, i.e. the ratio of oil (1.456) to that of  
243 the dispersion medium (1.33), was 1.095. The mean droplet size was reported as the  
244 Sauter-average diameter ( $d_{32}$ ) and the volume-average diameter ( $d_{43}$ ) from the size  
245 distribution results. Each individual  $d_{32}$  and  $d_{43}$  value was reported as the mean and  
246 standard deviation of at least three readings made on triplicate samples.

247 A zetasizer (ZS Nano, Malvern Instruments Ltd, Malvern, UK) was used to  
248 measure the electrophoretic mobilities of the MCNC-stabilised emulsion droplets.  
249 The diluted emulsion samples were transferred into DTS1070 folded capillary cells  
250 for measurement of the electrophoretic mobility, followed by 30 s of equilibration  
251 within the equipment. The mobilities were then converted to  $\zeta$ -potential values using  
252 the classical Smoluchowski equation. Each  $\zeta$ -potential value was reported as the  
253 mean and standard deviation of at least three reported readings made on triplicate  
254 samples.

255 The apparent viscosity, elastic modulus ( $G'$ ) and viscous modulus ( $G''$ ) of the  
256 emulsions were determined at 25 °C using a Kinexus ultra rheometer (Malvern  
257 Instruments Ltd, Malvern, UK). Steady shear experiments were performed; apparent  
258 viscosities as a function of shear rate in the range from 2 to 1000 s<sup>-1</sup> were recorded  
259 for the emulsions using a double gap geometry DG 24/27. Dynamic frequency  
260 sweep tests were then carried out at a strain amplitude of 1.0% and with an angular  
261 frequency range of 0.01–2.0 s<sup>-1</sup>. The frequency-dependent curves of the storage ( $G'$ )  
262 and loss ( $G''$ ) moduli were recorded. All measurements were done in triplicate and  
263 were reported as the mean and standard deviation.

264 CLSM images of the emulsions were taken using a Zeiss LSM 880 confocal  
265 microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany). Exactly 500  $\mu$ L of  
266 emulsion was added to a 1.5 mL Eppendorf tube and mixed with 10  $\mu$ L of Nile Red  
267 (0.1% w/v in dimethyl sulphoxide, excitation 514 nm, emission 539–648 nm) and  
268 then 100  $\mu$ L of Calcofluor-white (1.0% w/v in Milli-Q water, excitation 405 nm,  
269 emission 410–523 nm). The mixture was vortexed for 10 s and equilibrated for 10  
270 min, and then 30  $\mu$ L was placed on to a concave slide. The sample was fixed by  
271 adding 50  $\mu$ L of 1.0% w/w xanthan gum, was covered with a coverslip and was  
272 observed using a 63  $\times$  magnification oil immersion objective lens.

273

## 274 **2.6. Stability of MCNC-based emulsions under different stresses**

### 275 *2.6.1. Storage at 4 °C*

276 The emulsions E0.05, E0.10, E0.20, E0.50 and E1.00 were stored at 4 °C and were  
277 monitored using visual observation and droplet sizing and  $\zeta$ -potential measurements  
278 for 4 weeks.

## 279 2.6.2. pH and ionic strength conditions

280 Freshly prepared emulsions (E1.00) had a pH of approximately pH 6.8. For the pH  
281 study, the emulsions were adjusted to pH 7.0 using 1 N NaOH and then to pH 6.0,  
282 5.0, 4.0, 3.0 and 2.0 using 1 N HCl. For each pH study, 20 mL of emulsion E1.00 in a  
283 50 mL beaker was adjusted to the desired pH by drop-by-drop addition of NaOH or  
284 HCl under constant stirring at 500 rev min<sup>-1</sup>. In separate experiments, emulsions  
285 (E1.00) were adjusted to various ionic strengths from 10 to 150 mM NaCl by mixing  
286 10 mL of emulsion with a suitable quantity of solid NaCl under constant stirring at  
287 500 rev min<sup>-1</sup>. Subsequently, samples were analysed for any change in droplet size,  
288  $\zeta$ -potential and microstructure (CLSM). All measurements were done in triplicate and  
289 were reported as the mean and standard deviation.

290

## 291 2.7. Statistical analysis

292 Analysis of variance was conducted using [Minitab® version 17.3.1](#) to detect overall  
293 significant differences ( $p < 0.05$ ).

294

## 295 3. Results and discussion

### 296 3.1. Characteristics of MCNCs

297 We first discuss the modification of the CNCs using OSA and any effects of this  
298 hydrophobic modification on the particle behaviour and morphology, as measured for  
299 the CNCs and MCNCs with a range of complementary techniques, such as FTIR  
300 spectroscopy, XRD profiles, static water contact angles and TEM ([Fig. 1](#)). This sets  
301 the scene for understanding the behaviour of the MCNCs when they are present at  
302 an interface. As expected, the modification reduced the number of hydroxyl (–OH)  
303 groups in the cellulose backbone because of their replacement by OSA in MCNCs;  
304 this was clearly observed in the FTIR absorbance spectra ([Fig. 1A](#)). The intensity of  
305 the band at 3400 cm<sup>-1</sup>, which corresponds to the –OH groups, decreased by  
306 approximately 14% in the MCNC sample, which was in agreement with a previous  
307 study ([Chen et al., 2018](#)), in which the intensity of the same band also decreased  
308 because of the substitution by OSA. There was a significant increase in the band at  
309 1600 cm<sup>-1</sup>; this corresponded to an increase in the water content of the MCNCs and

310 was associated with the addition of some water molecules during the modification  
311 process, which was also observed in the previous study (Chen et al., 2018).

312 Modification with OSA also led to changes in the crystalline form (allomorph) of  
313 cellulose I, the diffraction pattern of which was between planes 101 and 002, as  
314 indicated in Fig. 1B (Ciolacu, Ciolacu, & Popa, 2011). There was an increase in the  
315 intensity of the peak between diffraction planes 101 and  $10\bar{1}$ , which indicated  
316 increased amorphousness or decreased crystallinity. This observation was in  
317 contrast to previous studies on the OSA modification of cotton CNCs (Chen et al.,  
318 2018) and the organic acid modification of bacterial cellulose nanofibres (Lee et al.,  
319 2011), in which the intensity of the peak between planes 101 and  $10\bar{1}$  decreased. In  
320 our study, the CIs of the CNCs and MCNCs were 77.7 and 68.8% respectively. That  
321 is, modification led to an around 11.5% decrease in the CI of the cellulose structure.  
322 This change was nearly three times higher than that reported by Chen et al., (2018)  
323 and Lee et al., (2011), which was around 4.5% and 5.0%, respectively. This  
324 discrepancy in the crystallinity of the MCNCs in the present study versus previous  
325 studies (Chen et al., 2018; Lee et al., 2011) might be attributed to the differences in  
326 source of CNCs used and the modification methods. For instance, Lee et al. (2011)  
327 used bacterial cellulose nanofibres while we used a commercial CNCs synthesised  
328 by sulphuric acid treatment of cellulose derived from bleached kraft pulp (Reid et al.,  
329 2017); both materials have different original crystallinity indexes (CIs), i.e. 90.2 and  
330 77.7 % for cellulose nanofibres and CNCs in the current study, respectively. The  
331 differences might affect their behaviour and reactions during the subsequent  
332 modification process. In the present study, we controlled the pH at a constant value  
333 for at least 7 h, which was an important factor in improving the degree of substitution  
334 of OH by OSA. In contrast, Chen et al., (2018) did not control the pH and mixing with  
335 OSA was carried out for 1 min only.

336 As a result of this OSA substitution, the hydrophobicity of the MCNCs was  
337 significantly enhanced; this was clearly demonstrated by the dramatic increase in the  
338 water contact angle from  $56.0 \pm 0.3^\circ$  (CNCs) to  $80.2 \pm 0.8^\circ$  (MCNCs) (Fig. 1C). The  
339 differences in the organisation of the crystal as shown in XRD data in Fig.1B upon  
340 modification with OSA might also explain the shifting of static water contact angle in  
341 Fig.1C towards more hydrophobicity. That is, MCNCs will be wetted preferentially by  
342 water and only partially wetted by the oil phase; thus, they will stabilise O/W

343 emulsions, as opposed to unmodified CNCs, which are too hydrophilic and will  
344 probably remain in the aqueous phase (Cherhal, Cousin, & Capron, 2016;  
345 Kalashnikova, Bizot, Cathala, & Capron, 2012). Changes in the surface charge of the  
346 CNCs were also determined, by comparing the  $\zeta$ -potentials of CNCs and MCNCs in  
347 1 wt% solution at pH 7.0. The CNCs had a high negative charge of  $-39.3 \pm 1.0$  mV  
348 because of the presence of sulphated groups in the cellulose backbones. In addition,  
349 the  $\zeta$ -potential ( $-39.1 \pm 2.1$  mV) of the MCNCs was similar to that of the unmodified  
350 CNCs. Although some changes in crystallinity were evident in the XRD profiles, the  
351 TEM images (Fig. 1D) of the CNCs and the MCNCs were indistinguishable, showing  
352 a needle-like shape and a similar aspect ratio (length:diameter) of 20:1, with a  
353 diameter of 2–4 nm and a length of 40–100 nm.

354

### 355 **3.2. Characteristics of MCNC-based Pickering O/W emulsions**

356 MCNCs at various concentrations (0.05–1.00 wt%) were used to produce 20 wt%  
357 Pickering O/W emulsions. As a control, emulsions were also prepared using 1.00  
358 wt% unmodified CNCs. Supplementary Fig. S1 confirms that the unmodified CNCs  
359 did not have emulsifying capacity; phase separation with a clear oil layer occurred  
360 almost immediately after preparation of the emulsions, and exceptionally large oil  
361 droplets were observed in the confocal micrographs. This is in agreement with the  
362 low water contact angle observed in Fig. 1C and again confirms the need for  
363 hydrophobic modification to enable the formation of stable oil droplets. The MCNC-  
364 stabilised emulsions were characterised by droplet size distribution (Fig. 2A),  
365 confocal microscopy (Fig. 3) and  $\zeta$ -potential (Table 1).

366 Fig. 2A clearly shows that all emulsions had bimodal distributions, with similar  
367 first peaks in the range 0.01–0.3  $\mu\text{m}$ . As the MCNC concentration increased, the first  
368 peak became more prominent and the second peak narrowed and shifted to a lower  
369 size range. In all emulsions, the first peaks were too small to represent emulsion  
370 droplets; they probably represented the MCNCs, which had a needle-like shape with  
371 a length of 40–100 nm (Fig. 1D). Hence, a possible explanation for the first peak was  
372 the presence of unadsorbed MCNCs in the aqueous phase. In a previous study on  
373 emulsions stabilised by CNC–protein composites, a similar small-sized peak was  
374 observed; it was hypothesised to be unadsorbed CNCs (Sarkar et al., 2018b). To  
375 prove this hypothesis, we centrifuged an emulsion at 14,500 g for 40 min at 4 °C,

376 collected the cream layer, diluted it in Milli-Q water to a droplet concentration similar  
377 to that of the original emulsion and analysed it for droplet size distribution. The  
378 droplet size distributions of emulsion E0.50 and the cream phase of emulsion E0.50  
379 confirm the hypothesis that the first peak was unadsorbed MCNCs, as it did not  
380 appear in the diluted cream phase in Fig. 2B. In addition, we also measured the  
381 particle size distribution of the MCNC solution using dynamic light scattering (DLS)  
382 (Supplementary Fig. S2). The MCNC solution had a multimodal distribution, in which  
383 the main peak was in the size range 100–300 nm, closely resembling that of the first  
384 peak of the droplet size distribution in Fig. 2A. Although the DLS size is in agreement  
385 with the characteristic length of the MCNCs (Fig. 1D), caution should be used in  
386 interpreting the DLS data, recognising the limitation that DLS assumes particles to  
387 be spherical; MCNCs are rod shaped (Boluk & Danumah, 2014). Therefore, in this  
388 study, the calculation of average particle sizes  $d_{32}$  and  $d_{43}$  were done after removal  
389 of the first peak in the range 0.01–0.3  $\mu\text{m}$  (the peaks were separated by a dashed  
390 line, see Fig. 2A) similarly to a previous study (Zembyla, Murray, & Sarkar, 2018). In  
391 addition,  $d_{32}$  and  $d_{43}$  of the first peaks of all the freshly prepared emulsions were  
392 quantified and presented in Supplementary Table S1. As can be clearly seen in  
393 Supplementary Table S1, the  $d_{32}$  and  $d_{43}$  of all the first peaks were around 33–38  
394 and 58–86 nm, respectively, which were within the range of lengths observed for the  
395 needle-shaped MCNCs (40–100 nm) TEM (see Fig. 1D). This analysis again  
396 confirmed that the first peak was due to the presence of free MCNCs in the  
397 continuous phase.

398 The results also confirmed the nature of the Pickering emulsions, in which the  
399 diameter of the droplets was around 10–100 times larger than that of the solid  
400 particles, which has been reported in many previous studies (Hu et al., 2015;  
401 Marefati et al., 2018; Sarkar et al., 2016). Furthermore, in our study, the average  
402 droplet size ( $d_{43} = 2.29 \mu\text{m}$ ) obtained at low MCNC concentration (0.20 wt%) was  
403 smaller than that of most Pickering droplets that have been reported (Li et al., 2018;  
404 Marefati et al., 2018; Tzoumaki et al., 2011; Yusoff & Murray, 2011). This indicates  
405 the excellent partial wetting of these MCNCs by either of the two phases.

406 Confocal micrographs of the Pickering emulsions are shown in Fig. 3, in which  
407 A represents the Calcofluor-white channel (MCNCs) and B illustrates the merge  
408 channel of Calcofluor-white and Nile Red (oil droplets). It can be seen clearly that

409 the amount of MCNCs in emulsion E0.05, with an oil volume fraction of 20 wt%, was  
410 not high enough to prevent coalescence. This is in agreement with a previous study  
411 on emulsion gels stabilised by OSA-modified CNCs, which reported substantial  
412 coalescence when low concentrations (less than 0.3 wt%) were used (Chen et al.,  
413 2018). However, the 'clear blue ring' in Fig. 3 (zoomed image) highlights the  
414 signature of the MCNCs acting as Pickering stabilisers. An increase in the MCNC  
415 concentration from 0.05 to 0.10 wt% resulted in a 1.97-fold decrease in average  
416 droplet size ( $d_{43}$ ) to 4.02  $\mu\text{m}$  (Table 1). In addition, the MCNC coverage on the  
417 interface was evident in the confocal images (Fig. 3), with smaller droplets being  
418 formed at higher MCNC concentration (0.05–0.10 wt%) and with a greater amount of  
419 unadsorbed MCNCs being present in the continuous phase, in agreement with the  
420 size distribution results (Fig. 2A).

421 Another study on 20 wt% O/W emulsions stabilised by carboxylated CNCs (0.1  
422 wt%) reported similar droplet sizes (Mikulcov, Bordes, Minarik, & Kasparkov, 2018)  
423 to those obtained in our study. However, in their study, the microscopic structure of  
424 the emulsions was not examined. In our study, there was significant improvement  
425 when the concentration of MCNCs was further increased to  $\geq 0.20$  wt%. Most  
426 droplets in these emulsions appeared to be fully covered by MCNCs and, even in a  
427 populated area, the droplets were well separated with distinctive layers of MCNCs on  
428 the interface without any sign of uncoated droplets. Also, there was no sign of  
429 droplet aggregation. This may have been associated with the higher negative  
430 surface charge ( $-68.7$  mV) obtained at higher MCNC concentration (Table 1), which  
431 prevented the droplets from coming in close vicinity of each other. In comparison  
432 with the surface charge of the aqueous dispersion of MCNCs ( $-39.1 \pm 2.1$  mV), as  
433 reported in Section 3.1, the surface charges of all emulsions were significantly  
434 higher. This might be due to the difference in the arrangement of the MCNCs  
435 between bulk solution and the oil droplet surface, which needs to be investigated in  
436 future using XRD. The MCNCs were more densely packed at the surface of the  
437 droplets than in the solution. A similar difference in particle charge between in the  
438 bulk phase and at the interface has been reported previously (Sarkar et al., 2016).  
439 Electrostatic repulsion is generally common in Pickering emulsions when the  
440 particles carry a surface charge (Araki, 2013; Binks, 2002; Ridel, Bolzinger, Gilon-  
441 Delepine, Dugas, & Chevalier, 2016). Therefore, the MCNCs allowed the formation

442 of Pickering O/W emulsion droplets that carried large desorption energies, because  
443 of their particle-stabilised interface, and at the same time were electrostatically  
444 stabilised, because of the high charge densities of the MCNCs at the interface.

### 445 **3.3. Stability of MCNC-based Pickering O/W emulsions**

446 The emulsions were also examined for their microstructural stability and their  
447 physicochemical properties when subjected to storage at 4 °C and to different pHs  
448 (pH 2.0–7.0) and ionic strengths (0–150 mM NaCl).

#### 449 *3.3.1. Storage for 4 weeks at 4 °C*

450 [Fig. 4](#) shows the appearance of freshly prepared emulsions and emulsions after 1, 2  
451 and 4 weeks of storage under refrigerated conditions. Apart from emulsion E0.05, in  
452 which a thin oil layer had separated from the remaining emulsion, all emulsions were  
453 stable, without any signs of creaming or separation. After 1 week of storage, there  
454 was clear phase separation in emulsions E0.05 and E0.10. Instability of the  
455 emulsions was due to there being an insufficient amount of emulsifier to stabilise all  
456 interfacial area at the chosen oil content (20 wt% oil).

457 This phenomenon was also in line with the CLSM images ([Fig. 3](#)), which  
458 showed large droplets in emulsions E0.05 and E0.10. In contrast, the droplet sizes of  
459 emulsions E0.20, E0.50 and E 1.00 changed slightly ([Table 1](#)) but the appearance of  
460 the emulsions was rather homogeneous. The  $\zeta$ -potentials of emulsions E0.20, E0.50  
461 and E1.0 remained constant after 1-week storage, suggesting that the high net  
462 negative surface charges were providing electrostatic repulsive forces to prevent  
463 flocculation. After 2 weeks of storage, emulsion E0.20 showed almost an order of  
464 magnitude change in droplet size. The zeta-potential of E0.20 significantly  
465 decreased, i.e.  $-60.9 \pm 2.6$  and  $-50.7 \pm 7.1$  mV after 1 and 2 weeks respectively  
466 ( $p < 0.05$ ). This was in agreement with increase in droplet size (3.55 times) and  
467 associated emulsion instability. Although creaming was clearly seen in both emulsion  
468 E0.50 and emulsion E1.00, both emulsions returned to a visually homogeneous  
469 appearance after a gentle shake, and there were no signs of coalescence. In  
470 addition, there was no significant change in droplet size and  $\zeta$ -potential in emulsions  
471 E0.50 and E1.00 after 4 weeks of storage.

472 In a previous study (Mikulcov et al., 2018), emulsions stabilised by less than 0.3  
473 wt% carboxylated CNCs showed clear separation of bulk oil from the remaining  
474 emulsion, indicating coalescence. In our study, for MCNC concentrations higher than  
475 0.20 wt%, creaming was detected only after 14 days. Overall, this suggests that  
476 0.50–1.00 wt% of MCNC was sufficient to create fine droplets with sufficient particle  
477 coverage, as shown in the confocal micrographs (Fig. 3), a high negative charge  
478 (Table 1) and resistance to coalescence over the storage period.

### 479 3.3.2. pH conditions

480 The emulsions subjected to different pH conditions (pH 2.0–7.0) were characterised  
481 using droplet sizing (Fig. 5A), flow curves (Fig. 5B) and confocal micrographs (Fig.  
482 5C), with mean sizes and  $\zeta$ -potentials being reported in Table 2. Fig. 5A and Table 2  
483 show that decreasing the pH from pH 7.0 to pH 5.0 slightly affected the sizes (1.3  
484 times) and net negative surface charge of the emulsions. However, when the pH was  
485 decreased to  $\leq$  pH 4.0, the droplet size increased significantly, by approximately 2.7  
486 times, and the magnitude of the  $\zeta$ -potential decreased from  $-30.4$  mV at pH 4.0 to  $-$   
487  $20.5$  mV at pH 2.0. In addition, when the pH was  $\leq$  4.0, the first peak of the size  
488 distribution decreased in width and the second peak shifted to a larger droplet size  
489 range.

490 For understanding of the role of MCNCs when present at the particle-  
491 stabilised interface, we first evaluated the effects of pH on the behaviour of aqueous  
492 dispersions of MCNCs (1.0 wt%) (Supplementary Fig. S3), which demonstrated that  
493 the  $\zeta$ -potential became significantly less negative as the pH was decreased from pH  
494 5.0 to pH 4.0. Behaviour of MCNCs in the aqueous dispersions and in the emulsions  
495 was not in complete agreement but the changes in  $\zeta$ -potential were similar within the  
496 pH range 4-5. This phenomenon might be due to the difference in alignment and  
497 concentrated coverage of the MCNCs at the emulsion droplet surface as compared  
498 to that in the bulk aqueous dispersions. In addition, as discussed in Section 3.2, the  
499 high surface charge of the emulsions contributed by the MCNCs would guarantee  
500 strong electrostatic repulsion to prevent droplet aggregation. At lower pH, the net  
501 negative charge was reduced, resulting in insufficient electrostatic repulsion to  
502 prevent closer approach of the oil droplets. As a consequence, there was droplet  
503 aggregation. The confocal images of the emulsion at pH 3.0 in Figs. 5C1 and 5C2

504 clearly illustrate emulsion flocculation. Aggregation of the emulsion droplets was  
505 associated not only with the MCNCs on the droplet surface but also with the  
506 unadsorbed MCNCs in the aqueous phase. This might explain the decrease in the  
507 first peak of the size distribution, which represented the unadsorbed MCNCs, as  
508 explained in [Section 3.2](#). Interestingly, there was no sign of coalescence in the  
509 emulsions on pH adjustment. That is, weak electrostatic interaction of the droplets at  
510 low pH induced aggregation but did not cause desorption of the MCNCs.

511 Studies on Pickering emulsions stabilised by CNCs from various sources have  
512 also reported the same trend, in which the changes in the magnitude of the  $\zeta$ -  
513 potential were a function of pH ([Liu et al., 2018](#); [Mikulcov et al., 2018](#); [Wen, Yuan,  
514 Liang, & Vriesekoop, 2014](#)). However, in these studies, the authors did not  
515 characterise the microscopic structure of the Pickering emulsions under various pH  
516 conditions.

517 For a better understanding of the effects of pH, the bulk rheological properties  
518 of the emulsions at pH 7.0 and pH 3.0, hereafter referred to as emulsion E7.0 and  
519 emulsion E3.0 respectively, were determined by applying shear rates from 2 to 1000  
520  $\text{s}^{-1}$  and then from 1000 to 2  $\text{s}^{-1}$  to the emulsions to observe hysteresis (if any) ([Fig.  
521 5B](#)). In general, the viscosity of E3.0 was higher than that of E7.0 due to droplet  
522 flocculation at lower pH. In addition, the viscosities of the emulsions at both pHs  
523 decreased as the shear rate increased within the investigated shear rate ranges,  
524 exhibiting a slight shear-thinning behaviour. Interestingly, both emulsions  
525 demonstrated negligible hysteresis within the range of shear rate investigated,  
526 indicating their stability during the shearing. In a previous study on Pickering  
527 emulsions stabilised by fumed silica, it was reported that the emulsions were stable  
528 against coalescence at a shear stress up to 1000 Pa and the emulsions reversed to  
529 their original state after the removal of the stress ([Lee et al., 2014](#)). However, this  
530 observation was in contrast to some other findings on Pickering emulsions stabilised  
531 by silica particles ([French, Taylor, Fowler, & Clegg, 2015](#); [Whitby & Krebsz, 2014](#);  
532 [Whitby, Fischer, Fornasiero, & Ralston, 2011](#)), where shearing from 0.1 to 1000  $\text{s}^{-1}$   
533 resulted in droplet coalescence ([Whitby, Fischer, Fornasiero, & Ralston, 2011](#)). In  
534 our study, no such shear-induced droplet coalescence was observed. In our study,  
535 emulsions E7.0 and E3.0 had  $\zeta$ -potentials of  $-64.3$  and  $-29.1$  mV respectively.  
536 Sufficient negative charge of the emulsion droplets possibly prevented the droplets

537 from coming in close vicinity and the higher mechanical strength of the MCNC-laden  
538 interface limited shear-induced coalescence.

### 539 3.3.3. Ionic strength conditions

540 On exposure to different ionic strengths (0–150 mM NaCl), the emulsions were  
541 assessed using droplet sizing (Fig. 6A) and apparent viscosity (Fig. 6B)  
542 measurements to understand the flocculation behaviour. In addition, the frequency  
543 dependence of the curves of storage modulus ( $G'$ ) and loss modulus ( $G''$ ) (Fig. 6C)  
544 together with their corresponding confocal images (Fig. 6D and 6E) gave an  
545 understanding of the ion-induced evolution in the material properties of these  
546 Pickering emulsions. The MCNC-stabilised Pickering emulsions were highly  
547 sensitive to changes in ionic strength at a critical electrolyte concentration (threshold)  
548 of  $\geq 20$  mM NaCl (Fig. 6A and Table 3). The volumetric proportion of the second  
549 peak increased significantly at the expense of the first peak. The average size  $d_{43}$   
550 increased significantly from 1.21  $\mu\text{m}$  in the absence of added NaCl to 2.04 and 76.86  
551  $\mu\text{m}$  at 20 and 100 mM NaCl respectively. Further increases in ionic strength did not  
552 lead to significant changes in the droplet size. In addition, the net surface charge of  
553 the emulsions decreased significantly from  $-64.3$  mV in the absence of added NaCl  
554 to  $-34.6$  and  $-18.6$  mV at 20 and 100 mM NaCl respectively (Table 3). It can be  
555 clearly seen that within the NaCl concentration range of 20-100 mM, addition of more  
556 electrolytes resulted in higher degree of reduction of net surface charge. This change  
557 was due to salt induced electrostatic screening of droplet charge and eventually  
558 formation of aggregates, as clearly observed in Fig. 6D and 6E.

559 For comparative purposes, the  $\zeta$ -potentials of aqueous dispersions of 1.0 wt%  
560 MCNCs at various NaCl concentrations (0–150 mM) were also determined  
561 (Supplementary Fig. S4). The net surface charge of the aqueous dispersion of  
562 MCNCs decreased as a function of NaCl concentration from 0 to 100 mM but a  
563 further increase in concentration did not lead to any significant changes in the  $\zeta$ -  
564 potential. This trend agreed with the effects of ionic strength on the surface charge of  
565 the emulsion E1.00 droplets. This indicates that the behaviour of the MCNCs in the  
566 bulk phase at various ionic strengths may also influence the behaviour of the  
567 MCNCs at the droplet surface. The addition of electrolytes above the threshold of 20  
568 mM NaCl will screen the charges associated with the cellulose molecules at the

569 surface. This might result in a reduction in electrostatic repulsion, and van der Waals'  
570 interactions might dominate, leading to aggregation (Boluk, Lahiji, Zhao, &  
571 McDermott, 2011; Chau et al., 2015; Prathapan, Thapa, Garnier, & Tabor, 2016;  
572 Zhong, Fu, Peng, Zhan, & Sun, 2012). Indeed, such aggregation of the MCNCs was  
573 evident in the confocal images of emulsion E1.00 at 20 mM NaCl (Fig. 6D). The  
574 decrease in the volumetric proportion of the first peak (Fig. 6A) was probably due to  
575 the utilisation of unadsorbed MCNCs to form bridges with the MCNCs adsorbed at  
576 the droplet surface, resulting in such aggregation. A further increase in the NaCl  
577 concentration to 100 mM led to the formation of a gel-like structure (Fig. 6E).

578 The apparent viscosities of the emulsions at 20 and 100 mM NaCl, hereafter  
579 referred to as emulsion E20 and emulsion E100 respectively, were also determined  
580 at shear rates from 2 to 1000 s<sup>-1</sup> (ramp up) and then from 1000 to 2 s<sup>-1</sup> (ramp down)  
581 (Fig. 6B). At both NaCl concentrations, the emulsions showed strong shear-thinning  
582 behaviour, with two orders of magnitude reduction in the apparent viscosity as a  
583 function of shear rate. This may have been due to reversible shear-induced  
584 breakdown of the flocs, supporting the gel-like behaviour observed in the confocal  
585 micrographs (Figs. 6D and 6E). The changes in the viscosity of emulsion E20 when  
586 the shear rate was ramped up and then down within the range 2–1000 s<sup>-1</sup> were  
587 identical, showing no hysteresis (Fig. 6B). In contrast, the viscosity curve of emulsion  
588 E100 (ramp up) should be taken with some cautions as the emulsion E100 showed  
589 wall slip, most likely due to the higher stress required to shear this sample (data not  
590 shown). Particularly, during the ramp down, the apparent viscosity was higher in the  
591 region 10–100 s<sup>-1</sup> but lower at 2–5 s<sup>-1</sup>. At a shear rate of 2 s<sup>-1</sup>, the apparent  
592 viscosity of emulsion E100 was about 50% lower at the end of the ramp down than  
593 at the start of the ramp up, largely associated with the slip, which leads to apparent  
594 decrease in the measured viscosity during ramp up (Franco, Gallegos, & Barn,  
595 1998). Such pronounced slip might be associated with the emulsion E100 forming a  
596 gel-like network at low shear rate. A similar phenomenon has been reported in  
597 previous studies (Kim, Song, Lee, & Park, 2003; Nandi, Khakhar, & Mehra, 2001;  
598 Schokker & Dagleish, 1998; Whitby et al., 2011). Strong shearing may destroy the  
599 gel network (Kim et al., 2003). Although at higher shear rates, 100 to 1000 s<sup>-1</sup> and  
600 the corresponding downward shear rate sweep test overlap, the contribution of slip  
601 cannot be neglected. Such flow behaviour of E100 needs further attention in future  
602 studies using rheometer geometries with ridged or roughened surfaces that are

603 designed to mitigate wall slip (Sánchez, Valencia, Franco, & Gallegos, 2001). In our  
604 study, emulsion E20 was partially aggregated, i.e. some droplets were still well  
605 separated. In addition, emulsion E20 had a relatively high negative charge (−34.6  
606 mV) (Table 3). In contrast, emulsion E100, as shown in Fig. 6E, was completely  
607 aggregated, meaning that the mobility of the droplets in the system was very  
608 restricted. Furthermore, emulsion E100 had quite a weak negative charge (−18.6  
609 mV), approximately half that of emulsion E1.00 in the absence of NaCl. Therefore,  
610 during the shearing, the ion-induced droplet network of emulsion E100 was prone to  
611 breakdown, caused by the shear-induced disruption of flocs, but the effect was  
612 largely reversible.

613 To understand the gel-like behaviour of these flocculated Pickering emulsions  
614 in the presence of ions, dynamic oscillatory measurements were performed (Fig.  
615 6C). The response of the Pickering emulsions to the applied frequency was  
616 determined at a constant strain (1.0%) within the linear viscoelastic region. The  
617 dominance of  $G'$  over  $G''$  in Fig. 6C convincingly confirmed the elastic gel-like  
618 structure of the emulsion at NaCl concentrations of  $\geq 20$  mM, which is in agreement  
619 with the network-like structure in Fig. 6E. Such observations can be attributed to the  
620 flocculation of droplets through the sharing of MCNC particles at the interface, as  
621 discussed above. The formation of an elastic network can also be explained by the  
622 tight packing of droplets that is associated with the charge screening by ions. As  
623 expected, the first peak in the size distribution almost disappeared when the NaCl  
624 concentration was  $\geq 100$  mM (Fig. 6A), which suggested that the unadsorbed  
625 MCNCs in the bulk phase associated strongly with the MCNCs at the interface,  
626 where the droplets behaved as 'active fillers' (Torres, Murray, & Sarkar, 2016).  
627 Interestingly, although sensitive to aggregation, the emulsions as a whole were  
628 resistant to coalescence. This might be associated with the fact that the rod-shaped  
629 MCNCs in the bulk phase had a strong tendency to collectively form a 'space-filling  
630 isotropic gel' (Oguzlua, Danumah, & Boluk, 2017) in the presence of ions that were  
631 somehow entrapping the droplets in a gel-like network, preventing further inter-  
632 droplet interactions.

633

## 634 4. Conclusions

635 This study showed that OSA-modified CNCs have excellent emulsifying capacity  
636 because of a significant increase in hydrophobicity, which resulted in partial  
637 wettability by an oil phase without the need for an additional surfactant. Pickering  
638 O/W emulsions (20 wt% oil) stabilised by 1.0 wt% MCNCs had very small droplet  
639 sizes (1.22  $\mu\text{m}$ ) relative to those in other studies, and they resisted phase separation  
640 for up to 4 weeks of storage under refrigerated conditions. The emulsions were  
641 sensitive to aggregation at  $\text{pH} < 4.0$  and at ionic strength higher than  $\geq 20 \text{ mM NaCl}$ .  
642 The formation of aggregates under these conditions was associated with a reduction  
643 in electrostatic repulsive forces between the droplets. These aggregated emulsions  
644 at low pH and high ionic strength responded differently under shearing conditions; in  
645 particular, emulsions at higher ionic strength ( $\geq 20 \text{ mM NaCl}$ ) had a prominent gel-  
646 like character. Nevertheless, the emulsions were strongly resistant to coalescence  
647 under all pH and ionic strength conditions investigated, which might be associated  
648 with the formation of a thick and dense layer of MCNCs around the oil droplets as  
649 well as with the MCNCs in the bulk phase forming strong bridges with the MCNCs at  
650 the interface, resulting in a gel-like network that reduced the mobility of the droplets.  
651 Such unique responsiveness of MCNCs to elastic gel formation at acidic pH and in  
652 the presence of ions might be applied in the design of emulsion systems to target the  
653 delivery of bioactive compounds. Gastric destabilisation might be limited because of  
654 the gel-like structure formed at low pH, and because of the unresponsiveness of  
655 these particle-laden interfaces to proteolytic enzymes in the gastric phase. Future  
656 studies will be focused on understanding the fate of MCNC-stabilised droplets with  
657 encapsulated short chain fatty acids in an in vitro gastrointestinal model, in which  
658 these Pickering emulsion droplets will be subjected to a complex milieu of pH,  
659 divalent ions and biosurfactants.

660

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669

## 670 **Appendix A. Supplementary data**

671 Supplementary data related to this article can be found at

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673

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