Supporting information

Nanoprecipitation to produce hydrophobic cellulose nanospheres for water-in-oil Pickering emulsions

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Abbreviations

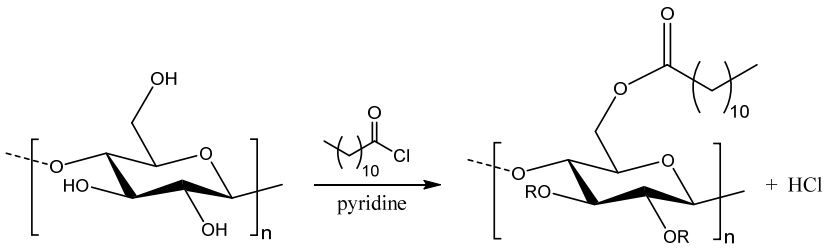
AGU	anhydrous glucose unit
DCM	dichloromethane
DI	deionized
DLS	dynamic light scattering
DS	degree of substitution
EC	esterified cellulose
ECS	esterified cellulose nanospheres
FTIR	Fourier transform infrared
MCC	microcrystalline cellulose
NMR	nuclear magnetic resonance
O/W	oil-in-water
RCF	relative centrifugal force
SEM	scanning electron microscopy
TGA	thermal gravimetry analysis
W/O	water-in-oil

1. Procedure for the synthesis of esterified cellulose (EC) and determination of the degree of substitution (DS)

a) Synthesis of cellulose esters

The cellulose esters were synthesized according to the general procedure described in in the main manuscript, Section 2.2 Esterification of MCC.

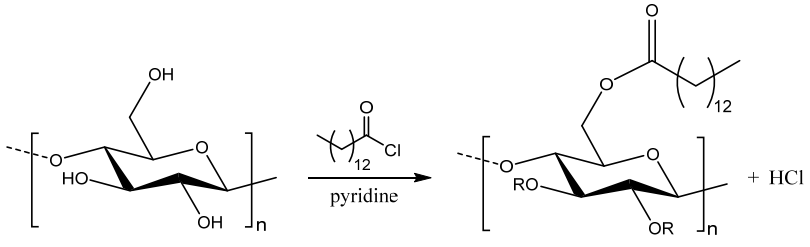
Cellulose laurate (dodecanoate, EC_C12)



R= $CO(CH_2)_{10}CH_3$ or H

Following the general procedure as described in Section 2.2 of the manuscript, to a suspension of MCC (500 mg, 3.1 mmol relative to AGU, 1.0 eq.) in dry pyridine (10 mL) was dropwise added lauroyl chloride (2.5 mL, 10.8 mmol, 3.5 eq.). The reaction mixture was stirred for 5 hours at 110 °C. The cellulose ester (1.2 g) was recovered with DCM (20 mL) as a white solid after solvent evaporation.

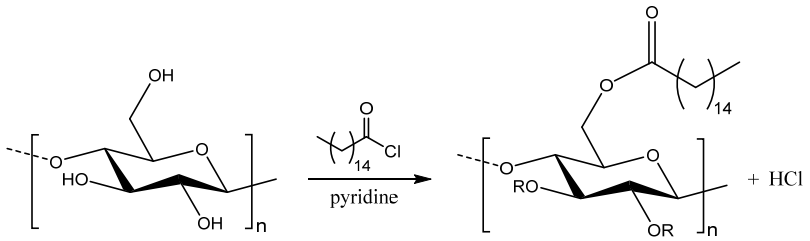
Cellulose myristate (tetradecanoate, EC_C14)



R= $CO(CH_2)_{12}CH_3$ or H

Following the general procedure as described in Section 2.2 of the manuscript, to a suspension of MCC (500 mg, 3.1 mmol relative to AGU, 1.0 eq.) in dry pyridine (10 mL) was dropwise added myristoyl chloride (2.9 mL, 10.8 mmol, 3.5 eq.). The reaction mixture was stirred for 5 hours at 110 °C. The cellulose ester (1.3 g) was recovered with DCM (20 mL) as a white solid after solvent evaporation.

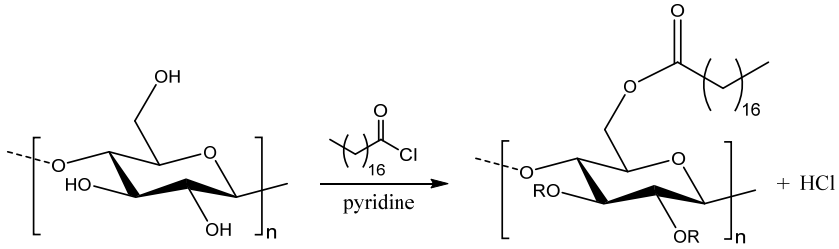
Cellulose palmitate (hexadecanoate, EC_C16)



R= $CO(CH_2)_{14}CH_3$ or H

Following the general procedure as described in Section 2.2 of the manuscript, to a suspension of MCC (500 mg, 3.1 mmol relative to AGU, 1.0 eq.) in dry pyridine (10 mL) was dropwise added palmitoyl chloride (3.3 mL, 10.8 mmol, 3.5 eq.). The reaction mixture was stirred for 5 hours at 110 °C. The cellulose ester (1.6 g) was recovered with DCM (20 mL) as a white solid after solvent evaporation.

Cellulose stearate (octadecanoate, EC_C18)



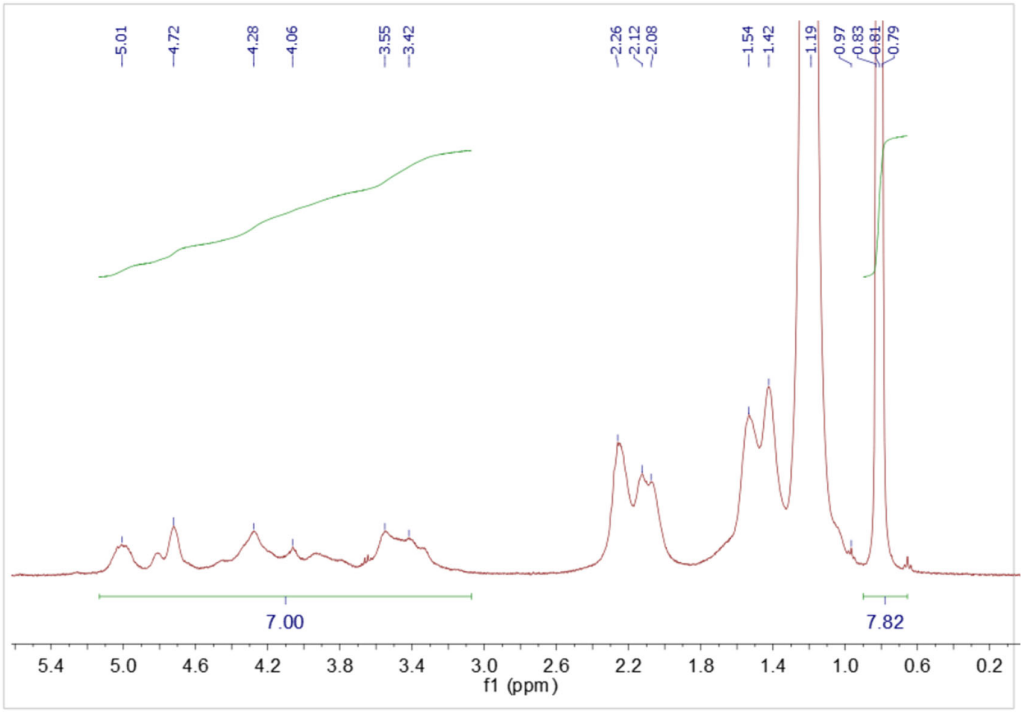
R= $CO(CH_2)_{16}CH_3$ or H

Following the general procedure as described in Section 2.2 of the manuscript, to a suspension of MCC (500 mg, 3.1 mmol relative to AGU, 1.0 eq.) in dry pyridine (10 mL) was dropwise added stearoyl chloride (3.6 mL, 10.8 mmol, 3.5 eq.). The reaction mixture was stirred for 5 hours at 110 °C. The cellulose ester (1.5 g) was recovered with DCM (20 mL) as a white solid after solvent evaporation.

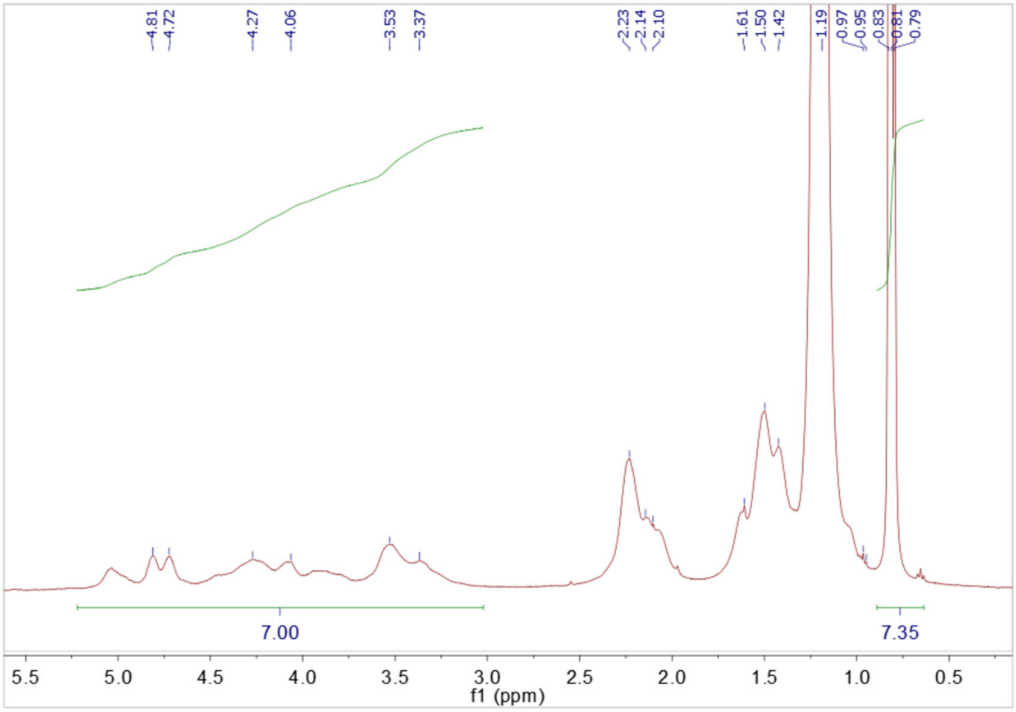
b) NMR Spectra for determination of the degree of substitution (DS)

The degree of substitution (DS) was calculated by working out the ratio between the integrations of the protons on the anhydrous glucose unit (AGU) and the protons on the terminal methyl group on the alkyl chain, obtained from the following ^{1}H NMR spectra as described in Section 2.6 of the main manuscript.

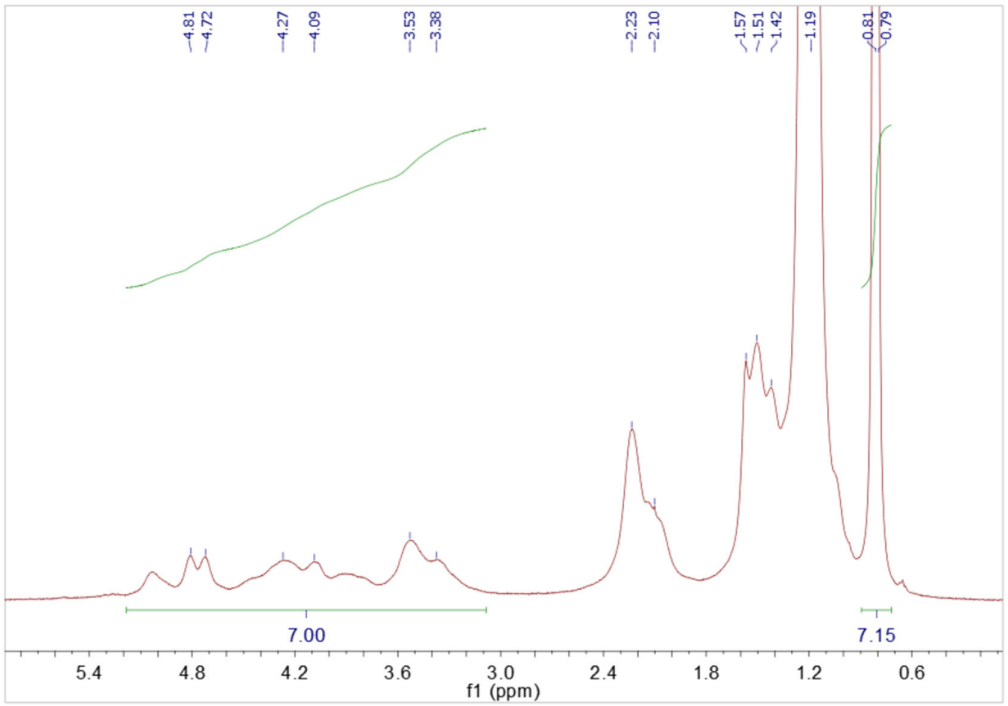
Cellulose laurate (dodecanoate, EC_C12)



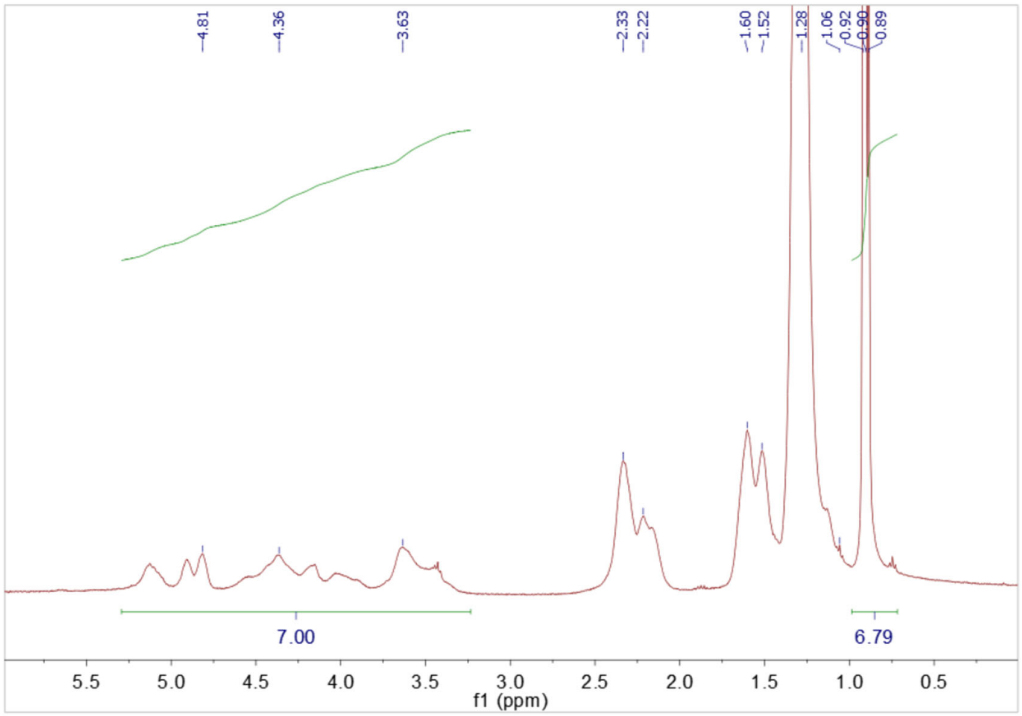
Cellulose myristate (tetradecanoate, EC_C14)



Cellulose palmitate (hexadecanoate, EC_C16)



Cellulose stearate (octadecanoate, EC_C18)

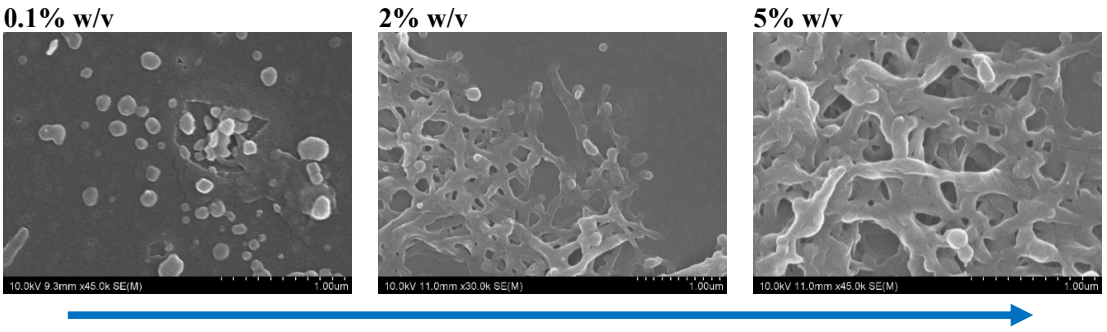


c) Degree of substitution for the esterified cellulose samples:

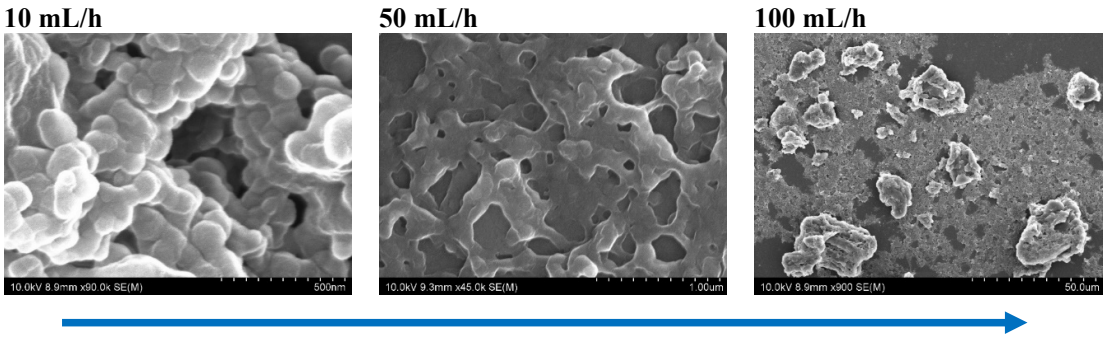
Esterified cellulose	AGU Integration	-CH_3 Integration	DS
EC_C12	7.0	7.82	2.61
EC_C14	7.0	7.35	2.45
EC_C16	7.0	7.15	2.38
EC_C18	7.0	6.79	2.26

2. Optimization of the nanoprecipitation procedure for formation of spherical nanoparticles

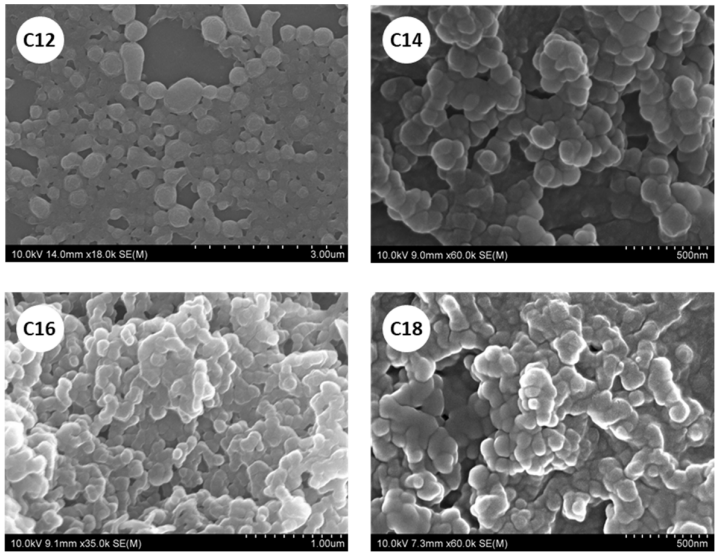
a) Below are shown representative images of the cellulosic structures obtained at different concentrations of EC in dichloromethane. The particle concentration played a crucial role in the formation of nanospheres as high concentrations favoured the formation of fibrils and extended networks. Concentrations need to be kept to below 1% w/v to promote the formation of nanoparticles.



b) Below are shown representative images of the cellulosic structures obtained at different rates of addition of a solution of esterified cellulose (EC) in dichloromethane. High addition rates of the cellulosic solution were found to be detrimental for the formation of nanospheres as rates higher than 20 mL/h induced the formation of nanofibril networks or microcrystals.



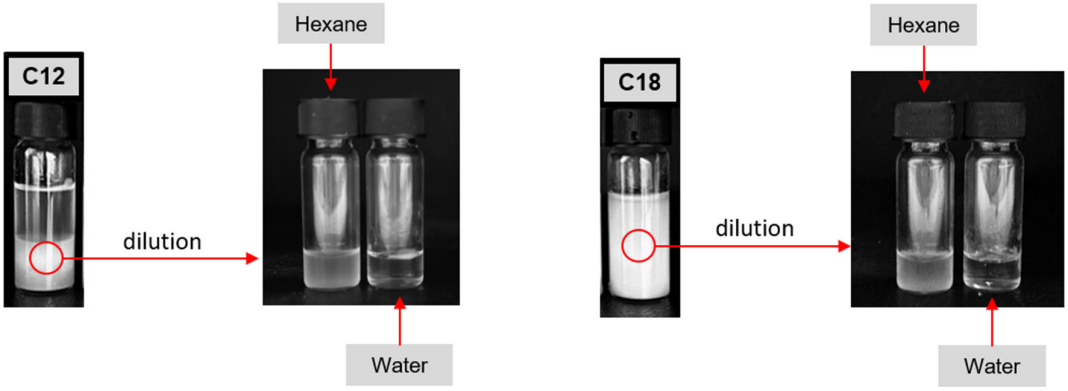
c) Representative images of SEM images of ECS_C12, ECS_C14, ECS_C16, and ECS_C18 formed using the optimized nanoprecipitation conditions (see Section 2.3)



3. Pickering emulsions with cellulosic derivatives

a) Pickering emulsions stabilized by esterified cellulose nanospheres (ECS)

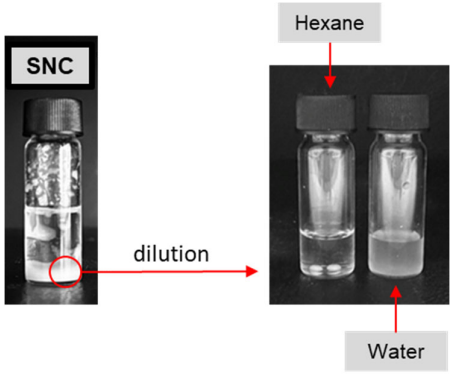
The type of Pickering emulsion (O/W or W/O) was determined using the drop dilution test (Gaukel, Schuchmann, & Bernewitz, 2015). Examples are presented below using the emulsions stabilized by the cellulose laurate (ECS_C12) and cellulose stearate nanospheres (ECS_C18), where we can see that the emulsions were dispersed in hexane rather than water, confirming that the dispersing medium for these emulsions is hexane.



b) Pickering emulsions stabilized by non-modified (hydrophilic) spherical cellulose nanospheres.

Hydrophilic spherical particles were synthesised by a nanoprecipitation technique based on the methodology presented by Zhang and co-workers (Zhang et al., 2019). Briefly, MCC was dissolved in an aqueous solution of NaOH (8% w/v), urea (8% w/v), thiourea (6% w/v), slowly precipitated in DI water under sonication, and separated by centrifugation. These particles were then investigated for their effectiveness in stabilizing Pickering emulsions.

The drop dilution test shown below indicates that the hydrophilic spherical cellulose nanoparticles formed O/W Pickering emulsions rather than W/O emulsions. In addition, poor stability was observed as the emulsion droplets sedimented immediately following emulsification.

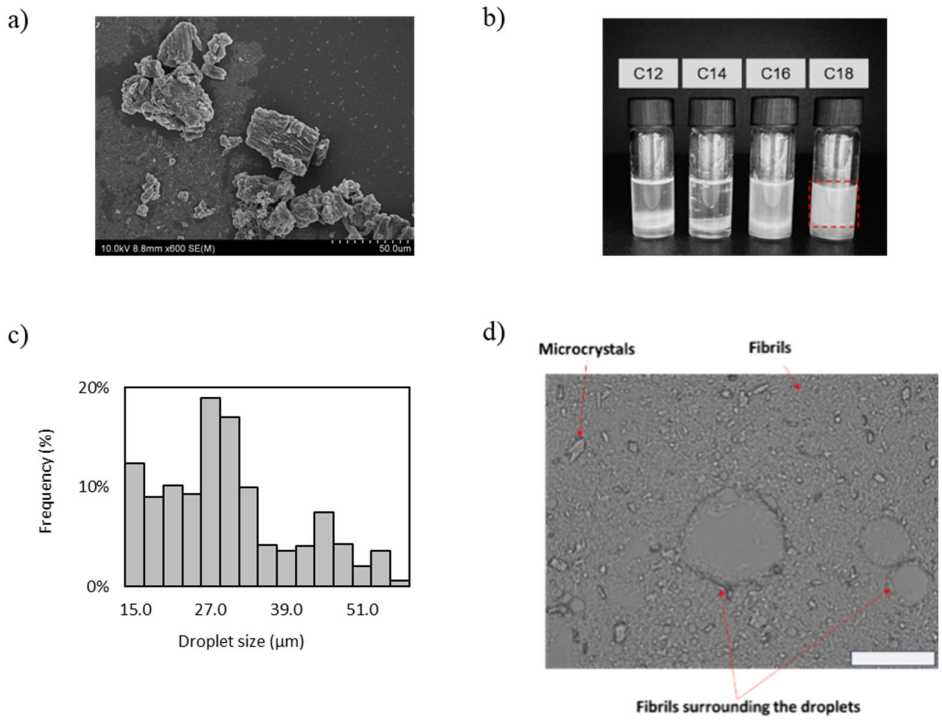


4. Emulsification with esterified cellulose (EC) before their nano-precipitation into nanospheres

The emulsion properties of the cellulose esters obtained before nanoprecipitation was analysed to understand the significance of forming nanospheres. Image (a) below shows an image of cellulose stearate (EC_C18) which appears as a combination of microstructures with a non-defined shape and non-uniform size distribution, which was also observed for the other cellulose esters EC_C12, EC_C14 and EC_C16. Perhaps the most interesting finding is that none of these cellulosic microstructures had the ability to form a single-phase emulsion at a concentration of 1% w/v (see image b, below) suggesting that the cellulose esters before the controlled nanoprecipitation exhibited lower performance in the stabilization of water in hexane emulsions even though they possess the same wettability. However, in accordance with what was seen with the nanospheres, the esters with longer carbon chains (i.e. EC_C18) was able to disperse a higher amount of water droplets in the hexane phase, showing some ability to stabilize the W/O emulsion.

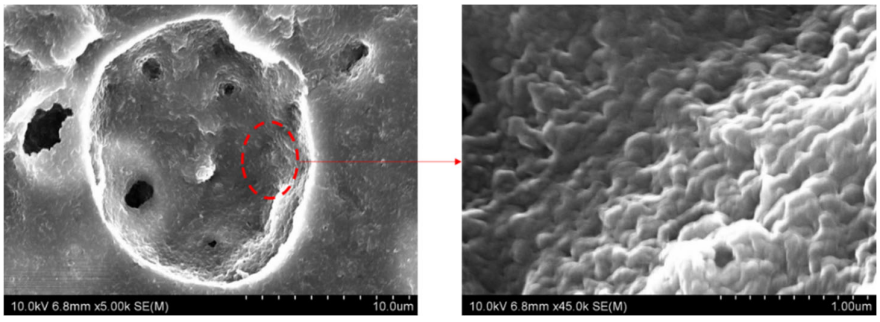
The size distribution of the droplets within the emulsions formed with EC_C18 shows a significantly larger average size of 28.5 μm (image c, below), which is more than four times higher than the size of the emulsion droplets formed with ECS_C18 (Figure 5e). The much larger size of the cellulose particles in EC_C18 is likely to be the key parameter determining the formation of the larger droplet sizes as several studies have shown a direct relationship between the particle size and the dispersed droplet size in Pickering emulsions (Sun et al., 2022). The

micrograph of the emulsion layer (image d, below) shows large droplets surrounded with fibrils and several dispersed cellulosic structures in the bulk medium suggesting desorption of the particles from the liquid-liquid interface due to phase separation. Overall, the low stability of the emulsions formed with EC_C18 provides rationale for the formation of particles in the nanoscale (ECS_C18).



6. Assembly of ECS_C18 at the hexane/water interface

The adsorption of the ECS_C18 at the hexane-water interface was confirmed by SEM as seen in the micrographs below. An emulsion droplet formed with ECS_C18 was directly imaged showing a round microstructure with a diameter close to the droplet averages determined in Figure 5e. Closer inspection of the droplet allowed us to identify the presence of ECS_C18 particles on the surface of the emulsion droplets.



7. References

Gaukel, V., Schuchmann, H., & Bernewitz, R. (2015). *Emulsion Characterization*. In *Encyclopedia of Membranes* (pp. 1-2)

Sun, Z., Yan, X., Xiao, Y., Hu, L., Eggersdorfer, M., Chen, D., . . . Weitz, D. A. (2022). Pickering emulsions stabilized by colloidal surfactants: Role of solid particles. *Particuology,* 64, 153-163.

Zhang, S., Zhang, F., Jin, L., Liu, B., Mao, Y., Liu, Y., & Huang, J. (2019). Preparation of spherical nanocellulose from waste paper by aqueous NaOH/thiourea. *Cellulose,* 26(8), 5177-5185.