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Drying of pickering emulsions in a viscoelastic network of cellulose microfibrils



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ABSTRACT

Oil-in-water Pickering emulsions at high volume fractions (50 wt % oil) are prepared using cellulose microfibrils (CMF) from plant cell wall materials as emulsifiers and stabilizer. Confocal microscopy is used to monitor the changing CMF network over time and the rate of oil droplet coalescence. Without addition of glycerol the drying emulsion quickly coalesces, and a significant oil separation ensues. The rate of coalescence and the percentage of coalescence droplets and oil separation decrease as the concentration of CMF is increased. Addition of glycerol into the emulsions decreases the extent of droplet coalescence and oil separation. At 10 wt% added glycerol, coalescence could not be prevented but oil separation is minimal: the oil is mainly contained into honeycomb shaped cells made of CMF and glycerol. The results demonstrate the influence of the aqueous phase on the coalescence and film forming properties of CMF stabilized Pickering emulsions.

1. Introduction

Concentrated oil-in-water emulsions are used in various products spanning different industries from the cosmetic and food industry to the pharmacy and fuel industry. They can resist shear stresses by behaving like elastic solids, even more so when solid particles are used to form Pickering emulsions [1,2]. Cellulose microfibrils can be used to stabilize oil in water emulsions, leading to micron sized oil droplets in a viscoelastic matrix of cellulose microfibrils (CMF) [3,4]. The CMFs form a shell around the oil droplets suggesting a Pickering emulsion type of

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stabilization, preventing the droplets from coalescing at room temperature when the oil droplets are sufficiently covered [5]. However, when a thin layer of said emulsion is dried on a substrate, the aqueous phase evaporates and the drying stresses lead to multiple coalescence events. The drying process of emulsions stabilized by surfactants has been studied in previous papers, but little is known about the drying kinetics of Pickering emulsions, especially of Pickering emulsions in a viscoelastic fibrillar network. For concentrated oil-in-water emulsions stabilized by surfactants, the drying process can be divided in 3 stages. First, the droplets come closer to each other as the water evaporates, until they reach close contact [6,7]. Then the droplets start to deform, leading to the formation of hexagonal droplets separated by a thin film of the aqueous continuous phase [8,9]. Finally, coalescence occurs, and formation of a macroscopic oil phase ensues.

It has been established that the evaporation rate of the continuous phase is one of the main factors influencing the drying kinetics and coalescence in surfactant-stabilized emulsions: slower drying rates are associated with higher critical disjoining pressures and less coalescence [10,11]. The surfactant concentration can also influence the disjoining pressure and the mode of coalescence [7]. In this paper we examine the drying stages of emulsions stabilized by CMF, and the influence of the continuous phase on the coalescence process. The properties of the continuous phase are varied by varying the CMF concentration, and by adding glycerol in the aqueous phase.

2. Materials and methods

2.1. Materials

Soybean oil was obtained from Sigma-Aldrich (Sigma Aldrich - S7381) and used as received (density = 0.9191 g/mL). Herbacel AQ + type N from Herbafood Ingredients GmbH Germany (84–90 wt % dietary fiber, 4–9 wt % water, 2–5 wt % ash) is used as cellulose raw material originating from Citrus peels (Lot number: 30902065) and used as received. It contains around 60 wt % of cellulose, 3.4 wt % of hemicellulose, and 5 wt % of proteinaceous materials [12]. Glycerol was obtained from Sigma-Aldrich (reagent grade, \geq 99%) and used as received. Deionized water was used throughout the experimental process.

2.2. Plant cell wall dispersion preparation

The plant cell wall powder is dispersed in water using a benchtop mixer (Silverson, USA) at 3500 rpm for 5 min, then passed through a high-pressure homogenizer with a Z-shape geometry at 1200 bar (Microfluidizer M 110S (MF), Microfluidics Corp., USA). The cellulose microfibrils come from the deagglomeration of plant cell walls when the dispersion in passed through the microfluidizer. The deagglomeration is such that very few cell intact cell fragments are present. Individual microfibrils measure about 3–4 nm in diameter, and they tend to form 10–25 nm bundles bound by the hemicellulose, pectins and proteins present in the peels [13]. More information can be found in the Supplementary material of our previous paper [5].

2.3. Emulsion preparation

Emulsions with different oil and CMF concentrations were prepared, namely 15, 30 and 50 wt% oil and 0.6, 0.9 and 1.2 wt% CMF. The oil is added to the CMF dispersion and mixed for 5 min at 3500 rpm using a benchtop mixer and passed through the homogenizer at 1200 bar. This yields stable emulsions with droplets sizes around 10 μ m in diameter [5]. As reported in our previous paper, the oil-in-water interface is stabilized by the proteinaceous materials bound to the CMF, so that the CMF forms a shell around the droplets preventing them from coalescing. The measured zeta potential is -51 mV, which is in line with the values obtained for similar emulsions stabilized by plant cell wall



Fig. 1. Scheme of the drying experiment chamber.

dispersions [14,15].

Glycerol is added gently to the system after the emulsification step at different concentrations in the final emulsion: 0.5 wt %, 1 wt %, 5 wt % and 10 wt %.

2.4. Drying experiment

Visualization of the emulsion microstructure was done with a confocal microscope Leica TCS-SP5 and DMI6000 inverted microscope (Leica GmbH, Germany). The CMFs were stained an hour before imaging the samples by adding 1 droplet of Direct Yellow (Solophenyl Flavine 96 at 0.5 wt%) to 1 mg of emulsion. The emulsions were injected into 20 mm wide and 0.6 mm deep round silicone isolators (Grace Bio-Labs, Sigma Aldrich) glued to a glass plate, and the drying process was monitored over time (Fig. 1).

Fluorescence images were obtained by excitation at 488 nm and emission at 496–555 nm. The drying front is followed as the emulsion thins down over time, and Z-stack images were taken just below the drying front. The resulting 1024×1024 images were then analyzed using the ImageJ software.

The surface area of the droplets was determined using the "Analyze particles" function of ImageJ and was used to estimate the local volume fraction ϕ from images of several hundred drops. As the droplets were compressed due to the water evaporation, they tend to have an elongated shape. The droplets were fit to an ellipse and the major axis *L* and minor axis *l* were determined using ImageJ. The aspect ratio of the droplets is calculated as *l/L*.

2.5. Coalescence frequency

Confocal micrographs with more than a thousand droplets are taken and the number of droplets is counted in each image using ImageJ. The percentage of coalesced droplets (N_{coal}) during the experiment is calculated with

$$N_{coal} = \left(1 - \frac{N(t)}{N(0)}\right) \times 100\tag{1}$$

With N(t) the number of droplets present at time t and N(0) the initial number of droplets before coalescence has occurred. N_{coal} equals 0 when no coalescence has occurred and approaches 100 as coalescence progresses. Multiple stacks of images are used to ensure reproducibility.

2.6. Separated oil determination

To calculate the amount of oil separation, the amount of oil that each sample lost to filter papers (55 mm diameter, Whatman) was determined at different time intervals when macroscopic oil separation was observed. The clean paper was weighed first, then was gently tapped on the surface of the drying emulsion and weighed again. The lost oil + paper weight was recorded. Oil loss was then calculated as



Fig. 2. Top: Dynamics of CMF rearrangement for an emulsion containing 50 wt % oil and 2 wt % CMF in the aqueous phase. Time intervals are 0 min (a), 10 min (b), 30 min (c), 60 min (d), 3 h (e). Scale bar 20 µm. Bottom: Graph showing the local volume fraction (in blue) and aspect ratio of the oil droplets (in green) as a function of time. Confidence intervals are drawn with translucent error bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the difference between the weight of the soaked paper and the weight of the clean paper, normalized by the sample weight. Measurements were made in triplicate. At all times, the filter paper relative to the sample size was large enough so as not to become saturated even on the longest measurement periods.

3. Results and discussion

3.1. Microstructure of the drying emulsions

Confocal microscopy images taken at different time intervals show the drying steps of an emulsion containing 50 wt% oil and 2 wt% CMF in the aqueous phase (Fig. 2(a–e)). The drying steps are similar to that of surfactant-stabilized emulsions. The oil droplets first come in close contact as the water evaporates, they then start to deform into hexagons as the emulsion become more compressed. As the water evaporates, the film separating the droplets becomes thinner and thinner, coalescence occurs and the excess particles in the water are trapped at the droplet surface. Coalescence progresses quickly until the oil physically separates from the CMF network, when the local volume fraction reaches ϕ = 80.5%.

During the first phase of the drying process when the droplets come close together, the aspect ratio does not vary while the local volume fraction of oil seems to increase slightly. The deformation of droplets from a spherical shape to a polyhedral one (Fig. 2b) does not have an impact on the aspect ratio, while there is a local increase in the concentration of oil due to water evaporation. The onset of coalescence begins at ϕ = 55% for this system as opposed to the maximum packing fraction of 74% for emulsion stabilized by surfactants [16]. In fact, the system reaches the maximum packing fraction at 55% already because of the presence of CMF bundles in the continuous phase that take up a significant volume.

During the coalescence phase, the aspect ratio of the droplets decreases dramatically, and the oil droplet size becomes very polydisperse (Fig. 2d). Many droplets do not relax into their original spherical shape. Instead they have an elongated shape due to the compression stress or arrested coalescence [16,17]. The oil volume fraction increases as well, and it can be seen from the confocal micrographs that the big CMF bundles in between droplets decrease in size. This is due to the contraction of the CMF bundles as the water evaporates. The concentration of CMF between the oil droplets increases as coalescence progresses.

When the local volume fraction reaches $\phi = 80.5\%$, the oil starts to separate from the samples and macroscopic pools of oil can be seen on top the emulsions. This layer of oil slows down the water evaporation rate and the coalescence rate. Coalescence progresses until the top of the sample is fully covered, at which point no change in microstructure was observed anymore in the entire emulsion. At the end of the experiment, a very open network of CMF with very large pores was obtained, the pores being filled with oil (Fig. 3b).

3.2. Influence of the cellulose microfibril concentration

From images sequences of the drying emulsions, the percentage of coalesced droplets was calculated using Eq. (1) and displayed on Fig. 4a.

The number of coalescence events is higher at low CMF concentrations, as shown by the starting slope of the curves. At low CMF concentrations the droplets start coalescing dramatically, and reach the plateau within 2 h, whereas at high concentrations the number of coalescence events increases steadily until the plateau 4 h later. The CMF form a shell around the oil droplets, so once full coverage is achieved, the remaining CMF create a viscoelastic network in the continuous phase [5,18]. The oil concentration is the same in all samples, so the amount of CMF required for emulsification should remain the same. The main difference is then the concentration of CMF in the continuous phase. Goel and Ramachandran [19] have shown that the film drainage process can be arrested when the film thickness between two droplets reaches a critical thickness h_f that is expressed as $h_f \frac{-6r^2R^3}{\gamma^2}$, with τ the yield stress of the continuous phase, *R* the radius of the droplets and γ the interfacial tension. Assuming that *R* and γ are the



Fig. 3. Confocal micrograph of an emulsion before drying (a) and after 5 h of drying (b) at room temperature. The emulsion contains 50 wt % oil and 1.2 wt % CMF in the aqueous phase before drying.



Fig. 4. (a) Percentage of coalesced droplets as a function of time for different CMF concentrations in the aqueous phase and 50 wt % of oil. (b) Volume fraction of oil that separated from the emulsions for different CMF concentrations in the aqueous and 50 wt % of oil. Confidence intervals are drawn with translucent error bands.

Table 1	
Initial composition of the emulsions studied.	

Glycerol added to the emulsions	φ _{oil} (wt %)	ϕ_{CMF} (wt %) in the aqueous phase	$\phi_{glycerol}$ (wt%) in the aqueous phase
0	50	1.2	0
0.5%	49.75	1.194	0.995
1	49.5	1.188	1.98
5	47.5	1.142	9.52
10	45	1.091	18.2

same for all systems at the onset of coalescence, the critical thickness is a square function of the yield stress of the continuous phase. The yield stress τ of the continuous phase increases with the CMF concentration [5] hence increases as more water evaporates. The critical thickness h_f then increases with the CMF concentration, explaining the lower initial coalescence rate. As coalescence progresses, the average droplet size and polydispersity increases. Hence, it becomes difficult to make estimations on the critical thickness. However, the model predicts that the percentage of coalesced droplets is dampened as the radii of the droplets increase, which is seen on Fig. 4a.

An increase in CMF concentration decreases slightly the percentage of coalesced droplets, this can be due to the combined effect of the increase in yield stress [19] and the decrease in the evaporation rate [10]. The distortion of the oil droplets and the presence of CMF in the aqueous phase result in a reduction of water transport, hence limiting the compression force required for droplets to coalesce. While the size of the shell of CMF around the droplets does not depend on the CMF concentration, the remaining CMF that has not adsorbed onto the droplets remain between the droplets, adding an extra layer of protection against coalescence. A thicker layer around oil droplets is typically associated with more mechanically stability and more resistance to deformation [1,20].

The plateau in the percentage of coalesced droplets is reached much sooner at low CMF concentration: very few coalescence events happen after 2 h of drying at 0.6 wt % CMF while it takes more than 4 h at 1.2 wt % CMF. Surprisingly, the volume fraction of separated oil has similar values at all CMF concentrations (Fig. 4b), indicating that it is not the oil separation that influences the coalescence rate at high CMF concentrations. One hypothesis could be that the additional biopolymers in the continuous phase contribute to the elasticity of the oil-water interface due to the interconnected CMF network, thus the droplets can sustain more deformation before film rupture and coalescence. Another one could be that since CMFs are hydroscopic, a higher concentration of CMF in the aqueous phase slows down the evaporation rate and slows down the drainage, hence limiting coalescence.

3.3. Influence of the addition of polyols

An emulsion containing 1.2 wt% CMF in the aqueous phase and 50 wt% oil is used and different samples with varying amount of glycerol are prepared by adding a certain amount of glycerol the emulsions. The composition of the emulsions before drying are shown in Table 1.

The initial oil content of the emulsions decreases a little when adding glycerol, but it doesn't influence the drying properties because the emulsion remains a concentrated emulsion. The CMF concentration in the aqueous phase decreases slightly as well. We have shown previously that a lower concentration of CMF in the aqueous phase leads to a higher percentage of coalesced droplets. However, as seen on Fig. 5, when 10 wt% of glycerol is added to the system, the emulsion still



Fig. 5. Dynamics of coalescence for a sample to which 10 wt % glycerol is added. Time intervals are 0 min (a), 30 min (b), 60 min (c), 2 h (d), and 4 h (e). Scale bar 20 μ m. (f) Droplet area distribution as a function of time, showing the difference between samples with and without glycerol. Colored bars show the interquartile range, the line across the bars indicate the median. End caps indicate the minimum and maximum droplet size detected.



Fig. 6. (a) Percentage of coalesced droplets as a function of time for different added glycerol concentrations. (b) Volume fraction of oil that separated from the emulsions for different added glycerol concentrations. Confidence intervals are drawn with translucent error bands.

coalescence but the resulting oil droplets are much smaller and less polydisperse. Coalescence leads to the formation of more or less regular honeycombs, with the CMFs located between the cells. Fig. 5f shows the droplet area distribution comparing a sample without added glycerol and with 10 wt % added glycerol. The sample without glycerol contains much bigger droplets that are more polydisperse. Very big droplets are detected after 2 h, this coincides with the beginning of oil separation. The resulting pool of oil (> 1 mm² area) is not considered in the droplet area detection hence the apparent reduction in droplet size. This does not happen when 10 wt% glycerol is added: the droplet area increases steadily but coalescence stops after 4 h.

The addition of glycerol seems to decrease the coalescence rate (Fig. 6a), the effect is especially dramatic when more than 5 wt% of glycerol is added to the emulsions. Coalescence does not progress as fast when glycerol is added, and less droplets coalesce together. The percentage of coalesced droplets after 5 h also decreases with added

glycerol. While coalescence could not be prevented for the studied samples, adding increasing amounts of glycerol limits oil separation, down to a few percent of separated oil when 10 wt % of added glycerol (Fig. 6b). As the percentage of coalesced droplets decreases with added glycerol, so does the droplet size and the probability of creating very large droplets.

The addition of glycerol in the emulsions led to a sharp decrease in the coalescence rate and oil separation. Two factors contribute to this decrease: the reduced water evaporation and the formation of a tighter CMF network. Glycerol is hydroscopic, so emulsions containing glycerol retain moisture and the water does not evaporate as fast [21,22]. It is likely that water remains in the CMF network even after 5 h drying, the amount of water trapped between the CMF probably increases with the amount of added glycerol. This added water content prevents catastrophic coalescence leading to oil separation. In addition, it has been shown that the addition of polyols in gelatin decreased the average hole size of gelatin matrices thanks to intermolecular interactions between water, glycerol and polymers at low hydration levels [23–25]. The effect was slightly stronger for glycerol than other polyols [26]. This is because the presence of glycerol lowers the inter an intra-molecular hydrogen bonding between cellulose molecules which improves the flexibility and chain mobility [27,28].

4. Conclusion

We investigated the drying of concentrated emulsions within a viscoelastic CMF matrix and the effect of CMF concentration and the nature of the solvent. Without the addition of glycerol, coalescence due to the drying stress lead the formation of very large oil droplets, eventually leading to a large oil separation, irrespective of the MCF concentration. Addition of glycerol to the emulsions limits the percentage of coalesced droplets and the oil separation, at 10 wt% a honeycomb structure is obtained, where the cells are filled with oil surrounded by CMF. The differences in the coalescence rate are attributed mostly to the decrease in water evaporation rate thanks to the higher CMF concentration and the presence of glycerol.

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