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On the stabilization of emulsions by cellulose nanocrystals and nanofibrils: Interfacial behavior and synergism

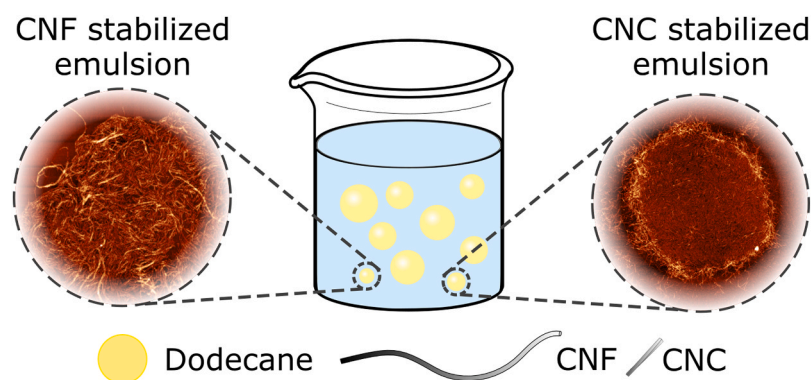
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GRAPHICAL ABSTRACT



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ABSTRACT

Competitive adsorption of cellulose nanofibers at oil-water interface in Pickering emulsion is reported here. Dodecane-in-water emulsions stabilized by either of two types of nanocelluloses, cellulose nanocrystals (CNC) or cellulose nanofibrils (CNF), as well as by their binary mixtures with increasing fractions of CNC, were prepared using particle concentrations of 0.1–0.5 wt% and studied. Despite differences in shape and morphology, both forms of nanofibers produced stable emulsion droplets even at low particle concentrations (0.1 wt%), with CNC producing smaller droplets and emulsions with higher stability. When mixed, an increased fraction of CNC in the mixture reduced the average droplet size, which however applied only for higher contents of oil (30 and 50 wt%) and higher total contents of cellulose particles used under emulsification. The CNC particles controlled the size of emulsion droplets, while the role of CNF contributed to the further surface coverage. When the fraction of CNF in the mixture increased, the capability of CNC particles to readily adsorb at the oil-water interface was reduced by the CNF nanofibrils present in aqueous phase. The stability of emulsions with respect to changes in droplet size and creaming index was influenced more by oil content and total particle concentration than by the fraction of CNC present in the mixture.

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1. Introduction

In recent years, particle-stabilized emulsions, also referred to as Pickering emulsions, have attracted both theoretical and commercial interest. As in many other applications, efforts in this field are currently directed towards the formulation of biocompatible colloids, and, thus, research into Pickering emulsions has recently shifted from using inorganic (especially silica particles) or petrochemical-based particular stabilizers to particles based on biopolymers. This important trend towards the use of environmentally acceptable products makes biodegradable and biocompatible materials, such as cellulose, starch, or lignin, increasingly attractive [1–5]. Cellulose nanomaterials possess these features have therefore become strong candidates due to their unique properties, such as their high aspect ratio, nanosize, biocompatibility, biodegradability, amphiphilicity, low toxicity, and renewability [6–8]. Cellulose nanoparticles and nanofibers, commonly referred to as nanocellulose, are composed of highly crystalline nanosized structures originating from parent microfibrils. Essentially, they can be classified into three main types – cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and microfibrillated cellulose (MFC), and bacterial cellulose (BC) – depending on the procedure used for their production from trees, plants, or other cellulose-containing species [9]. CNF and MFC share many structural similarities, even though they are made through distinct production routes. The preparation process and the sources of the particles also predetermine their physicochemical properties [10]. Cellulose nanoparticles have proven to be efficient in stabilizing O/W emulsions, as they fulfil the conditions of partial wetting for most oils, which facilitates their adsorption and favourable assembly at oil–water interfaces. Suitable surface modifications can produce hydrophobic cellulose nanofibers capable of forming W/O emulsions [11, 12].

Over the last few years, a number of scientific papers have described procedures for preparing Pickering emulsions stabilized by various grades of cellulose nanoparticles, such as CNC or CNF [13–15]. Under emulsification, CNC and CNF perform differently owing to distinct properties. CNCs are negatively charged, highly crystalline nano-rods obtained by acid or enzyme hydrolysis of the starting cellulose material, these ranging from 1 to 100 nm in diameter and from tens to hundreds of nanometres in length. CNF, on the other hand, is obtained via mechanical treatment of the starting materials and is composed of nanosized fibrils with diameters ranging from 10 to 100 nm and lengths of several tens of micrometres, depending on the source material [10]. In comparison with CNF, the behaviour of CNC particles as emulsion stabilisers is better described in literature and this cellulose type is also more frequently used for the preparation of Pickering emulsions. Previous works, for example, clearly documented that unmodified CNCs can efficiently stabilize emulsions and perform better when having a low surface charge density, or when the surface charge is screened by the presence of salts in the dispersion medium [16,17]. In contrast to emulsions based on CNC, CNF-based emulsions have received less attention and it was reported that, due to its very high aspect ratio, CNF forms in emulsions strongly entangled network. This stabilizing network is more evident at higher CNF concentrations and contributes to the overall stability of the emulsions [15]. Recently, a similar stabilization mechanism has also been reported for other types of cellulose nanofibers, such as bacterial fibrils. However, their effectiveness in stabilizing emulsions varies significantly as a result of differences in the diameters and aspect ratios of the fibrils [13,18].

Recently, Bai et al. have investigated the effect of combining cellulose nanofibrils and cellulose nanocrystals to prepare sunflower oil and dodecane emulsions. The authors focused on sequential or simultaneous addition of the nanocelluloses and on low volume oil fractions where the inter droplet interaction could be neglected and where creaming could easily take place. Under these conditions, the authors suggested that the emulsions are stabilized by depletion mechanism, above a critical CNF concentration. This was further evidenced with a concentration-

dependent behaviour. A very important aspect was that the simultaneous addition of CNC and CNF was a simpler choice for emulsification [19].

Here we explored similar concepts, however at much higher volume fractions of oil (up to 50 wt%), where the depletion mechanism may not be at play anymore and for which the adsorption of CNF at the oil/water interface may compete with the adsorption of CNC. The oil chosen was dodecane as it has proven to be a good model to study nanocellulose emulsification capacity. We also focused on longer storage time of emulsions (up to 9 months), as these appear also practically relevant. In this respect, the question whether CNC and CNF can cooperate and/or compete for space at the oil-in-water interface when used simultaneously is also discussed. Therefore, a series of samples stabilized with CNC/CNF mixtures containing different CNC wt. fractions were prepared and their properties in terms of droplet size, stability, and surface coverage studied.

2. Materials and methods

2.1. Materials

Nanocrystalline cellulose was obtained by acid hydrolysis of commercially available microcrystalline cellulose (Avicel PH101, FMC Biopolymer) according to the procedure described [20]. The average length and diameter of the nanofibrils were 234 ± 66 nm and 30 ± 7 nm, respectively, as determined by atomic force microscopy (AFM). Cellulose nanofibers were of gift of StoraEnso (Karlstad, Sweden). AFM analyses demonstrated the presence of long, strongly entangled nanofibrils ($> 1 \mu\text{m}$) of thickness greater than 30 nm. Dodecane from Sigma-Aldrich Co. (Steinheim, Germany) was used as delivered without further purification. Ultra-pure water was from a Mili-Q system (Merck, Darmstadt, Germany) and anhydrous calcium chloride was purchased from IPL (Uherský Brod, Czech Republic).

2.2. Preparation of Pickering emulsions

The Pickering emulsions were prepared with three different O/W ratios: 10/90, 30/70 and 50/50 (wt/wt). The aqueous phase consisted of ultra-pure water containing cellulose particles with total concentrations of 0.1, 0.3, 0.5 and 1.0 wt%. CNC and CNF were employed either individually or as mixtures with mass ratios of CNC/CNF of 5/1, 2/1, 1/1, 1/2, 1/5 (wt/wt). This corresponds to 0.83, 0.67, 0.50, 0.33 and 0.17 wt fraction of CNC in total cellulose amount (CNC+CNF). The mixtures of CNC/CNF were added to the aqueous phase simultaneously. In order to improve the emulsifying capacities of the systems, calcium chloride was added to the aqueous cellulose suspensions to a final concentration of 3 mM. Emulsifications were carried out by mixing dodecane (the oil phase) and the cellulose-containing aqueous phase using a Heidolph DX900 high-speed homogenizer (Heidolph Instruments, Germany). The homogenizer operated at 24 000 rpm for 5 min.

2.3. Size and distribution of emulsion droplets

The droplet size and droplet size distribution were measured using laser diffraction (Master Sizer 3000, Malvern instruments, UK). For these measurements, the emulsions were sampled and suspended in the instrument flow-system containing milliQ-water. The refractive index of the oil was set to 1.421. The volume mean diameter $D(4,3)$ corresponding to the mean diameter of spheres with the same volume as the analysed droplets was calculated according to $D(4,3) = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$, where n_i is the number of particles with diameter d_i . In addition, the Sauter mean diameter $D(3,2)$, used for determination of the surface coverage of emulsion droplets, was calculated according to the equation $D(3,2) = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}$ [21]. All analyses were performed in triplicates and are reported as means and standard deviations.

2.4. Microscopy

Emulsion droplets were observed using a Zeiss AxioCam MR 5 optical microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Prior to observation, tenfold-diluted emulsions were placed onto a glass microscope slide and viewed under 10–100 × magnification.

2.5. Atomic force microscopy (AFM)

AFM was used for the visualization of the dry residual of the emulsion droplet. After dilution of a ten-time fold with ultrapure water, 10 μL of the diluted sample was deposited on a freshly cleaved mica surface (Mica V-5 grade (SPI), size 10 × 10 mm). After 10 s of exposure to the diluted emulsions, the mica surfaces were dried with a stream of air. The dried droplets were visualized using a Ntegra-Prima AF microscope (NT-MDT). Images were recorded at a scanning rate of 0.5 Hz with a resolution of 512 × 512 pixels in tapping mode at ambient temperature. A silicone-nitride probe with a resonant frequency of 150 ± 50 kHz and a spring constant of 5.1 N/m (NSG01, NT-MDT) was used. Data were processed using Gwyddion 2.5 software (Czech Metrology Institute).

2.6. Emulsion stability

The creaming index (*CI*) was evaluated by visual observation immediately after preparation (1 d), after one week, and then after 9 months storage at ambient temperature. At regular time intervals, the height of the creaming/serum layers was measured and the *CI* calculated as $CI = (H_S/H_E) \times 100\%$, where H_E and H_S represent the total height of the emulsion in the tube and the height of the transparent serum layer, respectively [22]. In addition to *CI*, the diameter of emulsion droplets, *D* (4,3), was measured after 9 months storage at ambient temperature. All analyses were performed in triplicates and are reported as mean and standard deviation.

2.7. Surface coverage

The surface coverage (*SC*) was calculated according to Kalashnikova, Bizot, Cathala, & Capron [14,24] and Winuprasith & Suphantharika [15] using

$$C = \frac{m_p D_{3,2}}{6h\rho V_{oil}}$$

where m_p is the mass of adsorbed CNC (CNF or their mixtures), $D_{3,2}$ is the mean Sauter droplet diameter, h is the thickness of the adsorbed cellulose layer, ρ is the density of cellulose (1.6 g cm³), and V_{oil} is the volume of oil encapsulated in emulsion droplets. The calculations were performed using $h = 8$ nm for CNC and $h = 14$ nm for CNF [23]. For CNC/CNF mixtures with different CNC contents, the thicknesses of the stabilization layers were calculated on the basis of their compositions using the relative contribution of each of the particle types with respect to weight: $h_{MIX} = (CNC_{wt, fraction} \times 0.008) + (CNF_{wt, fraction} \times 0.014)$.

3. Results and discussion

3.1. Droplet size and distribution

The size of emulsion droplets is an important parameter with a key impact on the behaviour, properties, and stability of emulsions. It is worth noting that in the case of Pickering emulsions, the correlation between droplet size and stability does not always hold true, contrary to surfactant stabilized emulsions. In this work, the volume-weighted droplet diameter *D*(4,3) was used to evaluate changes in the samples. The values of *D*10, *D*50 and *D*90 given by diffraction measurements were also recorded; however, as they all followed the same trend, *D*(4,3) was adopted for discussion throughout the paper.

Emulsions stabilized solely by CNF or CNC contained droplets with sizes significantly influenced by the oil content, the type of cellulose particles, and the cellulose particle concentration (Fig. S1). In particular, it is clear that, for the same concentration of nanocellulose, the droplets stabilized with CNC were smaller than those stabilized with CNF, which was obviously because of the different morphologies and sizes of the stabilizing cellulose particles. CNCs are shorter in length, better cover the droplet surface, and form a flat monolayer at the oil-water interface thus facilitating the formation of smaller emulsion droplets [24]. In comparison, long and entangled nanofibrils of CNF are less able to adapt to a high curvature at the interface, which results in the formation of bigger droplets [15,24]. The droplets also become smaller with increasing cellulose concentrations, as more cellulose is available to stabilize a higher interfacial area formed by reducing the size of the emulsion droplets. This was mainly noticeable in emulsions with 30 and 50 wt% oil, in which an increase in the amount of stabilizing particles from 0.1 to 0.3 wt% caused an abrupt reduction in droplet diameter from about ~35 to ~15 μm (CNF emulsions with 30% oil and CNC emulsions with 50% oil). Bai, L. et al. [25], who studied CNC emulsions with 10% bioactive edible oils (including essential orange oil) prepared by microfluidization, observed a decrease in droplet size with increasing CNC concentration up to 0.75 wt% and a size-plateau at concentrations from 0.75 to 2 wt%. In the case of CNF, results reported in the literature do not agree. For instance, Winuprasith, T., & Suphantharika, M. [15] reported that an increase in CNF content from 0.05 to 0.7 wt% was responsible for an increase in droplet size in soybean emulsions. Here we observed the opposite as already shown in other reports [14]. We can infer that, unless the bulk viscosity is affected substantially by the type of CNF used which in turn would affect the emulsification process as such, the current observation of a decrease of droplet size when CNF concentration increases is expected as reported for many other colloidal systems used as emulsifier.

Another factor which had an impact on the droplet size of emulsions was the oil fraction; as it increased, the droplet size increased. This trend was observed for emulsions stabilized with both CNF and CNC. While with an oil fraction of 10 wt% the concentration of cellulose particles affected the droplet size only marginally, with oil fractions of 30 and 50 wt% the effect was pronounced.

Information on the relative fractions of droplets of different size in the emulsions was obtained via assessment of the droplet size distributions. The recorded distributions followed two main types: 1) symmetrical, unimodal distributions, which represent nanocellulose-stabilized emulsion droplets and 2) bimodal or asymmetric distributions with a main peak and a tail/small peak in the smaller size region. Here, the main population represents the emulsion droplets, and the second small fraction represents free, non-adsorbed nanocellulose particles/particle clusters.

Emulsions stabilized solely with a single particle type, CNC or CNF, exhibited prevalently symmetrical, unimodal distribution curves. Bimodal or asymmetrical distributions were observed only in samples with a low oil content and high cellulose concentrations. The bimodality was most apparent for CNC emulsions with 10 wt% oil and 0.3–1 wt% CNC, which is likely due to the better stabilizing properties of CNCs, as reported earlier by us [26] and others [27], these leading to smaller amounts of CNC than CNF being consumed to cover the interfacial areas of the oil droplets. The remaining free CNC then gave rise to the second peak in the distribution curve, which grew in size with increasing CNC concentration (Fig. S2). In general, the shapes of the distribution curves for CNC- and CNF-stabilized emulsions did not notably change when using 0.3–1.0 wt% nanocellulose. The relative independence of the droplet size distributions on the amount of nanocellulose particles was earlier observed by F. Jiang and Hsieh [28] and Gestranius et al. [13]. In contrast, at 0.1 wt% cellulose, the curves for both CNF and CNC emulsions were clearly shifted towards larger droplets. On the other hand, the increase in the oil fraction resulted in changes in the distribution curves for both CNC and CNF emulsions. For example, for CNF, Fig. S3 illustrates the presence of a symmetric and narrow distribution with an oil

content of 30 wt%, where the cellulose particles are mostly consumed in the process of stabilizing the interfacial area of oil droplets, and free cellulose is not present in the emulsion. The samples with lower (10 wt %) oil fractions, however, exhibited asymmetric distributions with notable tailing towards the small-size region.

Emulsions stabilized by mixtures of CNC/CNF followed a trend similar to the samples formulated with single particle type, and with the increase of total cellulose concentrations from 0.1% to 0.5% their droplets become smaller (Fig. 1). Cellulose concentrations of 0.3 and 0.5 wt% worked well for the production of all emulsions with 10–50 wt % oil, the former showing droplets with the smallest $D(4,3)$. Additional increase in cellulose concentration to 1 wt% did not further influence the droplet size; on the contrary, droplets of some of the emulsions grew slightly. The reason for this might be the presence of an excess of particles in the aqueous phase, which raises its viscosity, thus lowering the efficiency of emulsification [15]. A decrease in the total cellulose concentration down to 0.1 wt%, however, led to a significant increase in droplet size in emulsions with the higher 30 and 50 wt% oil fractions. With an oil fraction of 10 wt% and cellulose concentrations of 0.3 and

0.5 wt%, all mixtures performed similarly and yielded droplets of comparable size – i.e., of about 8–9 μm .

The effect of the CNC fraction in the cellulose particle mixture (CNC+CNF) on the droplet size $D(4,3)$ is also given in Fig. 1. With an increasing oil fraction, the better encapsulating properties of CNC over CNF are more evident and emulsions containing a higher amount of CNC yielded droplets with smaller diameters, as illustrated by the photomicrographs in Fig. S4 (a, b). In cases where the lowest concentration of nanocellulose (0.1 wt%) and the higher oil contents (30, 50 wt%) were combined, and, simultaneously, the amounts of CNC in the mixture decreased (CNC fractions 0.17 and 0.33), the diameter of droplets increased notably in comparison with samples where the contents of CNC were high (0.83). With respect to the influence of oil content, the emulsions stabilized with CNC/CNF mixtures behaved similarly to single-particle systems and higher oil fractions yielded bigger droplets. This is illustrated in Fig. S4 (c, d), with emulsions containing 0.5 wt% of cellulose particles in total with a CNC wt. fraction of 0.5. Here, the droplet diameters were 8.5 ± 0.01 and 20.8 ± 0.3 μm for 10% and 50% oil contents, respectively.

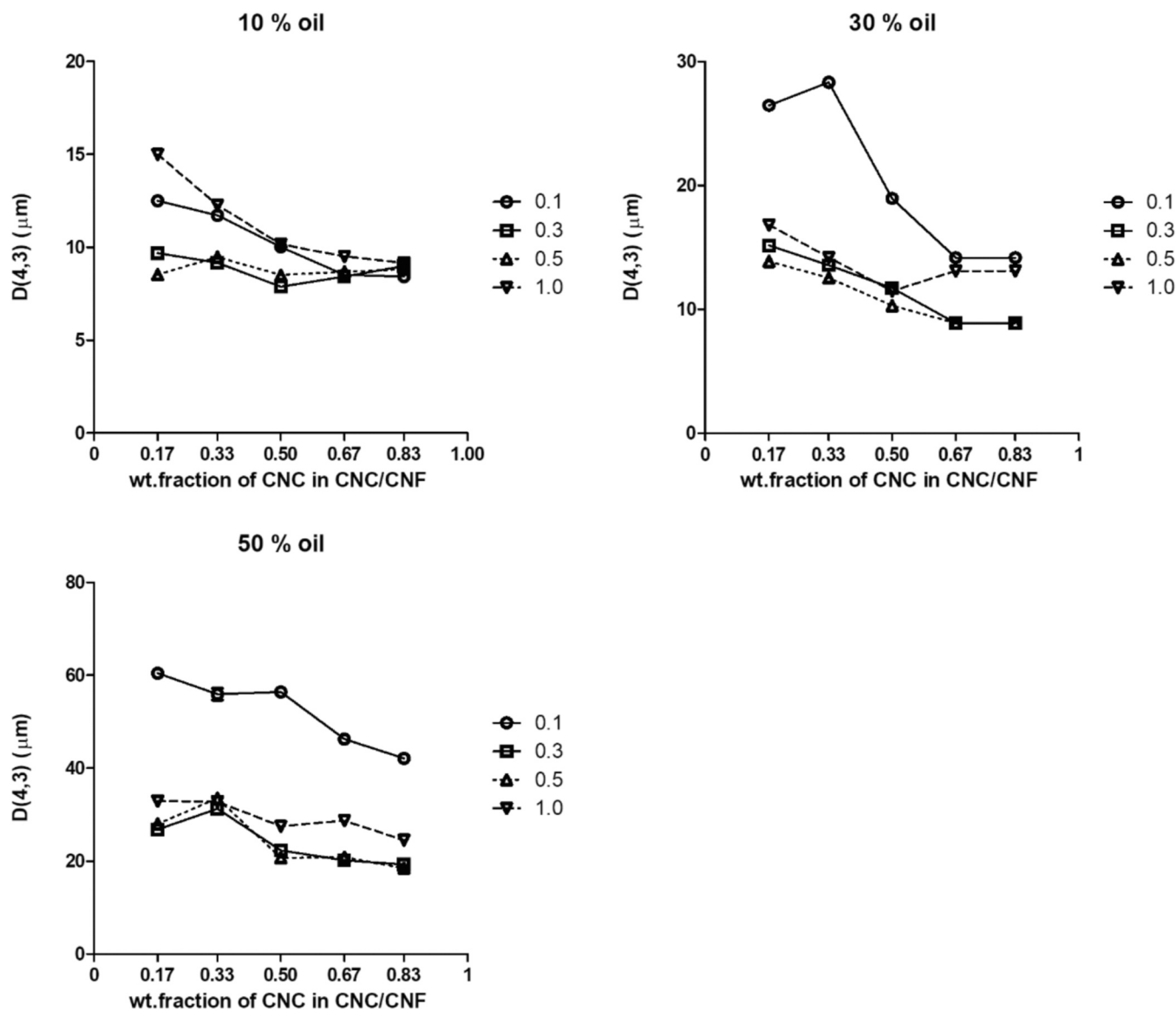


Fig. 1. Dependence of droplet size on wt. fraction of CNC in the CNC/CNF mixture recorded for emulsions stabilized with total particle concentration 0.1, 0.3, 0.5 and 1 wt% CNC/CNF. Oil content was of 10, 30 and 50 wt%. The error is ± 1 μm .

The CNC/CNF ratio also played a crucial role in controlling the droplet-size distribution in emulsions stabilized with mixtures of particles. Some of the emulsions contained unconsumed CNC free in bulk. This was mostly visible for emulsions with 10 wt% oil and mixtures with higher CNC fractions (0.83 and 0.67). With a low CNC fraction in the mixture, a bimodal distribution typically occurred in emulsions stabilized with high total cellulose contents, mainly 0.5 and 1 wt%. It can be suggested that the small peak at the two highest cellulose contents could be due to free CNF particles or their mixtures with CNC present in the aqueous phase. The excess of free CNF in emulsions prepared with these CNC/CNF mixtures can originate from the already-mentioned ability of CNC to better encapsulate dodecane droplets and preferentially adsorb at the interfaces, unabsorbed CNF thus remaining in the bulk. Interestingly, at higher fractions of CNF (CNC fractions of 0.17 and 0.33) and a 1 wt% total cellulose concentration, the distributions also contained a third peak/tail in the higher-droplet size region, likely composed of large CNF/CNC aggregates. The influence of the CNC/CNF ratio on droplet size distributions became, however, less important as the oil fraction increased, and emulsions containing 50 wt% oil were all monomodal, irrespective of the total cellulose concentration used.

3.2. Emulsion stability

The ability of emulsions to resist coalescence was assessed via changes in their droplet sizes after storage at ambient temperature for 1 week and 9 months. The emulsions prepared with CNC or CNF were stable throughout the storage time, with only minor changes observed for samples containing 30 and 50 wt% oil stabilized with 0.3 and 0.5 wt % nanocellulose, irrespective of its type. The droplets size increased when prepared with 1 wt% particles, which is likely because of the contribution of the inter-particle network formed from the excess cellulose in the aqueous phase, leading to potential flocculation. Fig. 2 (top) shows changes in $D(4,3)$ measured after storage relative to the initial size of the droplets, in percent. A significant increase in droplet size was observed in emulsions with 10 wt% oil (at all cellulose concentrations), with a higher coalescence observed for CNF emulsions (from 8 to 17 μm) in comparison with samples stabilized with CNC (from ~ 9 –13 μm); here the samples prepared with 0.5 wt% cellulose serve as example. The good resistance of CNF-stabilized emulsions to coalescence was reported earlier [29], this effect was ascribed to the presence of long CNF fibrils and the network formed thereof. At higher CNF concentrations, the emulsion stability can also be improved thanks to the increase in viscosity in the aqueous phase, which hinders the droplets from approaching and merging.

A certain similarity can be found between the behaviour of emulsions stabilized by individual particle types and their mixtures, as illustrated in Fig. 2 (bottom) (30 wt% oil) and Fig. S5 (10 and 50 wt% oil). Also, here, the samples with 30 and 50 wt% oil were reasonably stable, mainly in the presence of 0.3 and 0.5 wt% cellulose. At 0.1 wt% cellulose, however, the droplets size increased to larger extent than for emulsions with a higher CNC fraction in the mixture. This was most evident in emulsions with 30 wt% oil and to some extent with 50 wt% oil. Again, the predominance of CNC at the interface while CNF increased the bulk viscosity can explain this observation. With an additional increase in cellulose concentration to 1 wt%, the droplet sizes remained almost unchanged. Pickering emulsions normally exhibit much better stability towards droplet coalescence than emulsions stabilized with classical amphiphilic surfactants. The reason is the irreversible adsorption of stabilizing particles at the oil-water interface and the steric repulsion of emulsion droplets [30]. The instability of emulsions in terms of coalescence was low throughout almost one year of storage, which conforms to the generally accepted view that particle-stabilized emulsions show excellent resistance towards coalescence [31].

Along with coalescence, creaming also occurred. The creaming index (CI) showed variations in the course of emulsion creaming, being mainly governed by the oil and total cellulose contents (Fig. 3). All emulsions

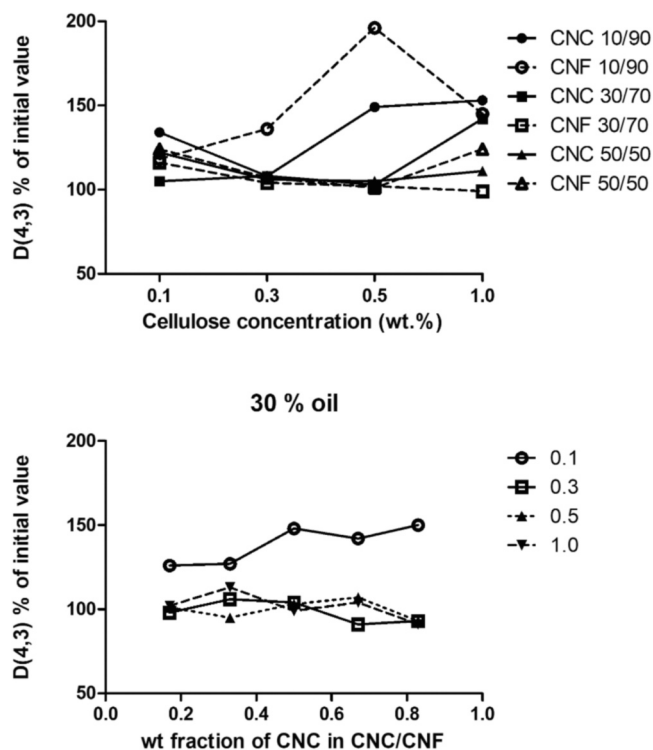


Fig. 2. Long term stability of emulsions a) stabilized with single particle type, CNC or CNF containing 10, 30 and 50 wt% oil; b) stabilized with 0.1%, 0.3%, 0.5% and 1% CNC/CNF mixtures of different wt. fraction CNC at fixed oil content of 30 wt%. The results are expressed as changes in $D(4,3)$ measured after 9 months storage relatively to initial values. The error is $\pm 5\%$.

stabilized with 1 wt% cellulose showed an absence of creaming, irrespective of the compositions of the CNC/CNF mixtures and the content of oil. At lower concentrations of cellulose (0.1 and 0.3 wt%), the creaming was more pronounced, mainly in emulsions with 10 wt% oil, as it was also reported by Míkulcová et al. [32]. Fig. 3 illustrates that higher fractions of the short, needle-like CNC particles in the CNC/CNF mixture (0.67, 0.83) reduced creaming, mainly when the total cellulose concentration increased. Visually, emulsions containing 50 wt% oil were the most stable, with a total absence of creaming at 0.5 and 1.0 wt % cellulose. Furthermore, the respective CI values for pure CNC and CNF systems show that both single-particle emulsions creamed less than those stabilized with mixtures, and that the creaming in CNC stabilized samples was lower than for the samples stabilized by CNF.

For the formulations prepared with 0.1 wt% cellulose, CI values correlated reasonably well with the sizing measurements. In contrast, creaming occurred in emulsions with 30 wt% oil stabilized with 0.3 and 0.5 wt% cellulose with no changes in $D(4,3)$. Here, flocculation may also occur without being detected by changes in $D(4,3)$, as the droplets loosely connected in flocks will be redispersed during sizing measurements in the continuously-stirred measuring cell of the diffraction instrument. Droplet flocculation induced by CNF was observed by Bai et al. [19], as mentioned earlier.

3.3. Composition analysis of the type of nanocellulose at the droplet surface

In the context of using mixed systems to stabilize emulsions, and understanding competitive adsorption, one important element is to be evaluated, at least qualitatively, is the composition at the droplet surface. Even though AFM is not the optimal method for visualizing emulsion droplets because of possible changes induced by drying on the mica surface, it can still illustrate the presence of different types of

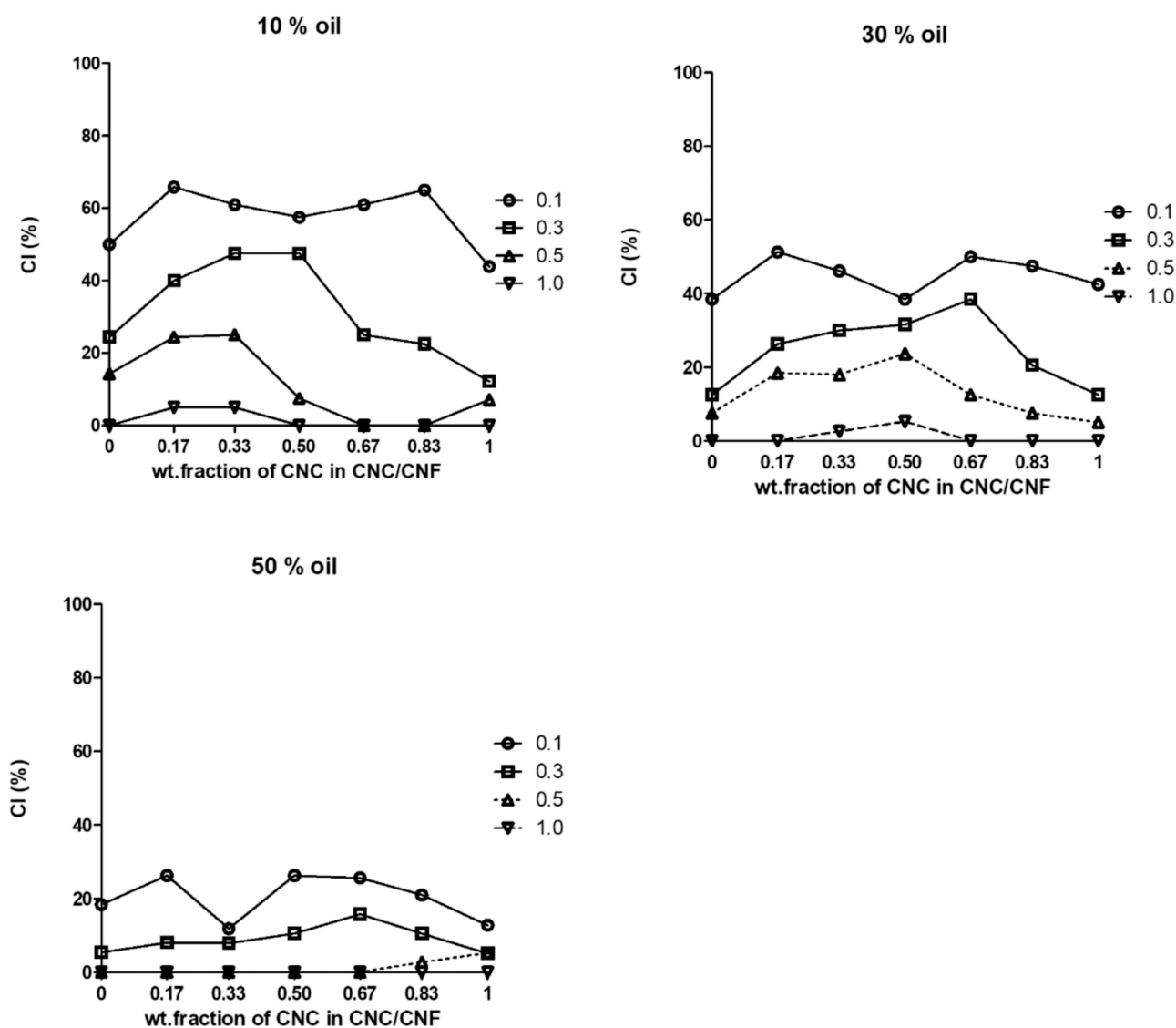


Fig. 3. Creaming index (CI) of emulsions prepared with a) 10 b) 30 and c) 50 wt% oil. Influence of CNC wt. fraction in CNC/CNF mixture and total cellulose concentration (0.1, 0.3, 0.5 and 1.0 wt%) is also included. The error is $\pm 2\%$.

cellulose particles at the oil-water interface. In the present case, we used emulsion that were diluted ten-fold and focused on dried droplet after deposition on a mica surface. Of course, with such a stressing process, the shape of the droplet cannot be retained.

The dried CNC droplet (0.5 wt% CNC) in Fig. 4a) shows a clearly visible stabilizing layer composed of needle-like nanocrystals neatly organized at the surface of the collapsed droplet. Also, the stabilizing layer does not exhibit a strictly monolayer character, which might be due to the Marangoni effect under the drying of emulsion droplets prior to AFM analysis, together with a loosely connected layer of CNC particles around the stabilizing CNC layer. In addition, free CNC particles are visible around the droplet, evidencing thus excess CNC in the aqueous phase for emulsions with 0.5 wt% cellulose. In contrast, Fig. 4b) shows tightly-packed and entangled CNF nanofibrils, and, unlike their shorter branches, the longer parts of nanofibrils seem to be embedded or partially embedded in the stabilizing layer. In this respect, important variations in shapes and sizes of nanofibrils within individual CNF particles can be noted, which can be a key factor in their organization at the interface. The appearance of a collapsed droplet stabilized by a CNC/CNF mixture (0.5 wt% CNC; Fig. 4c) is somewhat surprising, as the surface seems to be covered mostly by long CNF nanofibrils. This could support the hypothesis concerning competitive adsorption between CNC

and CNF. When present in mixtures in the aqueous phase, CNC adsorbs preferentially at oil-water interface followed by CNF, which long nanofibrils are therefore more visible after the droplets are dried on the mica surface.

3.4. Discussion and surface coverage analysis

Surface coverage (SC) was determined from the amount of particles involved in the stabilization of emulsions, the size of emulsions droplets ($D_{3,2}$), and the volume of encapsulated oil (Table S1) [19]. In these calculations, the free cellulosic particles are neglected. The evolution of the mean Sauter diameter of droplets ($D_{3,2}$) plotted against SC at varying concentrations of CNC or CNF (Fig. 5), together with stability data show the ability of even the lowest used nanocellulose concentration to sufficiently encapsulate oil.

When using 0.1 wt% CNC, SC ranged from 80% (10 wt% oil) to 44% (50 wt% oil); correspondingly CNF yielded SC of 77% and 35% for emulsions with 10 and 50 wt% oil, respectively. Even the observed SC values were significantly lower than 100%, the coverage was still sufficient to produce stable emulsions. This observation conforms with literature reporting a minimum SC of 44% to yield stable emulsions with CNC, while SC of 35% was needed for CNF [14,16].

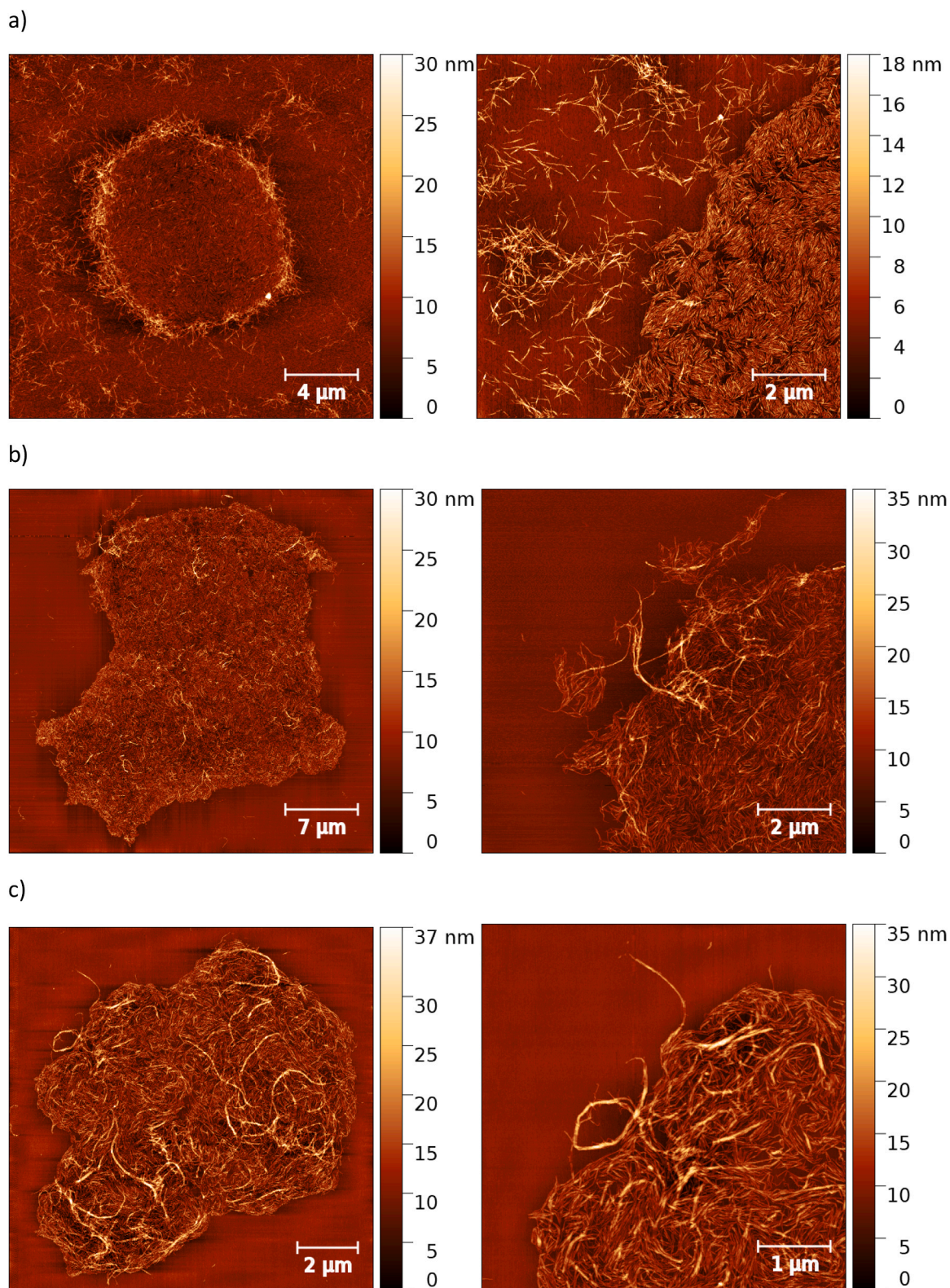


Fig. 4. Visualization of emulsion droplets using AFM after drying on mica support. Stabilization with a) CNC, b) CNF and c) CNC/CNF mixture with 0.5 CNC wt. fraction.

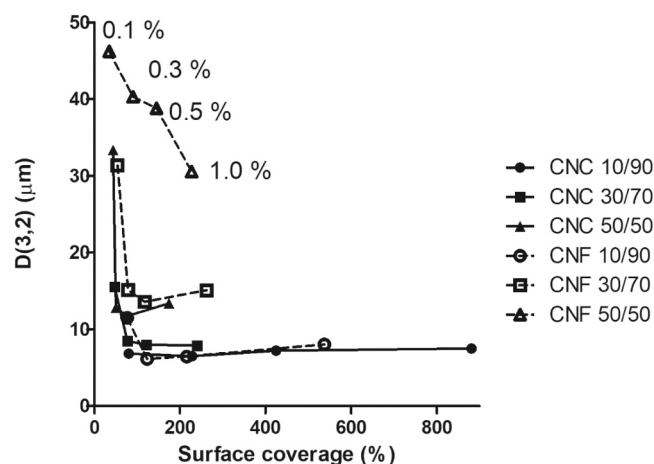


Fig. 5. Correlation of droplet size with surface coverage at varying contents of CNC and CNF for fixed oil contents of 10, 30, 50 wt% and at varying nanocellulose concentrations of 0.1, 0.3, 0.5 and 1 wt%, represented by each of the points of the respective curve.

With increasing cellulose concentration, SC increased notably, but the increase was not accompanied by a corresponding decrease in $D(3,2)$, indicating that at a given energy input, the size of the emulsion droplets did not decrease further, and that the amount of cellulose was sufficient to cover the droplets already formed.

Fig. 5 further illustrates that, with two exceptions, the $D(3,2)$ vs SC dependence followed a similar trend for all prepared emulsions, specifically a decrease in $D(3,2)$ with increasing SC up to a critical limit, followed by a plateau in $D(3,2)$ values. This behavior might be due to several effects, and the formation of multiple layers, particle rearrangements at the oil-water interface, and the formation of aggregates can be cited as possible reasons. In this respect Kalashnikova et al. [14] reported on the unlikely formation of multiple stabilizing layers on similar systems, as the cellulose particles irreversibly adsorb at the oil-water interface, which results from the principle of Pickering stabilization relying on partial wetting. However, Bai et al. [19] recently reported on the adsorption of CNF on CNC particles determined by the QCMD technique, and this finding keeps the possibility of the formation of stabilizing CNC/CNF multilayers still open. The first of the above-mentioned exceptions was exhibited by all CNC emulsions with 10% oil, these showing only a constant value of $D(3,2)$ with increasing SC. This is certainly the result of an excess of stabilizing CNC particles relative to the total surface area of the droplets formed from the available oil. CNF emulsions with 50 wt% oil were the second exception, showing a slow gradual $D(3,2)$ decrease with the absence of a plateau and reaching maximum SC of about 200% at the highest used cellulose concentration (1 wt%). This behavior provides unambiguous evidence of the greater ability of CNC to cover the oil-water interface and stabilize emulsions. In this respect, it is worth mentioning the denser organization of CNC at the oil-water interface, leading to a better coverage of emulsion droplets by CNC than CNF. Evidence for the formation of a denser, less permeable coverage layer on droplets stabilized with CNC has been reported by several research groups [12,24,26]. In the case of CNF-stabilized emulsions, the high surface coverage can be attributed to additional stabilization mechanisms typical for longer fibrils – specifically, to the formation of an interconnected network in the aqueous phase [13,19]. This also supports the possibility of having complementary depletion stabilization as observed previously [19]. All in all, it is difficult to only take into account one single mechanism, and it is likely that several effects are at play, one of them being the dominating one in that regime of oil concentration.

Surface coverages were also calculated for all emulsions stabilized with CNC/CNF mixtures at different oil and cellulose concentrations and

varying CNC fractions in the mixture, see Fig. S6, Table S2. The results indicated that the CNC fraction in the mixture affected the SC, however the oil fractions and total cellulose concentrations also played an important role. The SC for emulsions with 10% and 30% oil stabilized with 0.1% total cellulose content showed a decrease with increasing CNC fractions in the mixture. This behavior was also valid for samples with 30 wt% oil when using 0.3% and 0.5% particles, which might indicate a gradually increasing involvement of CNF in the stabilization of droplets. With an additional increase in total cellulose concentrations, the impact of the CNC fraction decreased and the SC values became higher than 100%, which points to an excess of particles in the samples. Values of SC calculated for the samples with 50 wt% oil and 0.1% cellulose were almost similar irrespective of the CNC fraction and ranged from 42% to 48%. Emulsions stabilized with 0.3% and 0.5% cellulose behaved similarly, though their SC were higher and the impact of the CNC fraction on SC was, in these cases, negligible.

The above-described behaviors of emulsions give highlight concerning the role of the different types of cellulose particles in Pickering stabilization. Certainly, both CNC and CNF, either alone or in combinations, are efficient particle stabilizers capable of forming stable emulsions. However, each of the particle types contributes to the stabilization process by means of different mechanisms. CNF acts through the morphology/shape of its nanofibrils (several micrometers in length) and, in addition to its stabilizing role, it influences creaming and/or emulsion stability either by inducing the depletion of flocculation, or, in increased amounts, by forming a 3D stabilizing network. The stabilization mechanism of the needle-like CNC particles lies in their capacity to adsorb quickly at the oil-water interface. The total cellulose content available also plays an important role.

When added under the form of a mixture, it is reasonable to assume that CNC, thanks to its greater mobility and the more uniform composition of its surface, reaches preferentially the interface, followed with a fraction of CNF participating together with CNC on the stabilization of the droplets. The remaining fraction of CNF then stays free in the bulk and its amount depends on the oil fraction and the total cellulose content in the emulsion. An increasing CNF concentration can, moreover, hinder the free movement of CNC particles in the bulk, influencing thus the ratio of CNC and CNF adsorbed at the oil-water interface and competition between CNC and CNF for space at the interface. Once adsorbed, CNC will remain at the interface, as the replacement of CNC by CNF at the interface is unlikely. One would expect that the bigger CNF particles would provide the better steric stabilization of oil droplets, as the longer fibrils can form a thicker protective layer. However, the fibrils can simultaneously interconnect the surfaces of two or more droplets and bind them together, which can lead to flocculation/coalescence giving rise to creaming [19]. In the aqueous phase, the situation is complicated by the presence of short CNCs, which might become entrapped within CNF nanofibrils, thus contributing to the formation of an interconnected structure leading to an increase in aqueous phase viscosity and emulsion stability. It should be mentioned that with respect to the above results we do not consider that the emulsification process plays an influential role here. In our study, the emulsification time was relatively short and the shear stress applied was low.

Other parameters can also affect the behavior at interface, especially during the emulsification process. CNC and CNF have different charge densities, around 250 $\mu\text{Equiv/g}$ [33] and around 25 $\mu\text{Equiv/g}$ [34], respectively. CNC particles have a specific surface area of about 154 m^2/g while longer CNF fibrils give a value of 148 m^2/g . This implies that electrostatic interactions between the CNC are certainly more important than in case of CNF. Tests conducted with and without the addition of salts for CNF showed no difference in its emulsifying properties, whereas CNC emulsions were negatively influenced by the absence of salts. This might indicate that in a mixed emulsion system, a certain amount of salts is consumed by CNF particles. As a result, a lack of salts to screen out negative charge might affect the positioning of CNC at the interface, leading to lower surface coverage.

Another possibility explaining the higher SC when higher CNF fractions are involved could be related to differences in the sizes and shapes of the particles. In terms of particle size, Matos et al. [33] proposed that larger particles, in our case CNF, can better stabilize mixed systems thanks to the following phenomena. Firstly, smaller particles experience a smaller convective transport force towards the interface, which in some cases may not be enough to overcome the barrier to adsorption, which corresponds to the minimum curvature of the particles. Secondly, small particles cannot benefit from capillary forces promoting particle packing at the interface.

4. Conclusion

The study presented here shows clear differences in the capacities of CNC and CNF to organize themselves at interfaces and stabilize oil-water emulsions with dodecane oil phase. Despite differences, both types of nanocelluloses produced stable emulsion droplets even at low particle concentrations, these droplets differing only in size, with CNC affording smaller droplets and more stable emulsions. When used in mixtures, an increased fraction of CNC reduced the average droplet size, which, however, applied only for higher contents of oil (30 and 50 wt%). This decrease in droplet size was supported by the positive influence of the increased total contents of cellulose particles used under emulsification. CNC was more efficient in transporting itself quickly to the interface, but only in cases when it was not hindered by an increasing amount of CNF in the aqueous phase. Therefore, CNC particles controlled the size of emulsion droplets through diffusion-driven processes, while the role of CNF lay in the stimulation of surface coverage, as its percolation limit was shifted to lower concentrations in comparison with CNC. However, when the fraction of CNF in the mixture was high, the capacity of CNC particles to adsorb at the interface significantly declined, as CNF nanofibrils increased the viscosity of the aqueous phase through entanglements. The stability of emulsions with respect to changes in droplet size and creaming index was influenced more by oil content and total particle concentration than by the fraction of CNC present in the mixture. As a result, we can conclude that the simultaneous addition of the two different types of cellulose particles, CNC and CNF, affords stable emulsions and that by varying the CNC/CNF ratio at a given oil content we can control the size and stability of emulsion droplets, which will allow for the preparation of “tailor-made” emulsions. Future work will lean towards potential nanocellulose surface modifications to tune the CNF-CNF interactions and adsorption behaviors. It would also be of interest to evaluate if nanocellulose shows the same behaviour with other oils such as triglycerides.

CRedit authorship contribution statement

Veronika Mikulcová: Conceptualization, Investigation, Methodology, Data curation, Writing. **Lucie Urbánková:** Conceptualization, Investigation, Methodology, Data curation, Writing; **Věra Kašpárková:** Conceptualization, Methodology, Writing. **Romain Bordes:** Conceptualization, Methodology, Writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2023.131975.

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