Cellulose nanofibrils for one-step stabilization of multiple emulsions (W/O/W) based on soybean oil

Carlos A. Carrillo a,b,⇑, Tiina E. Nypelö a,⇑,1, Orlando J. Rojas a,c,d

a Department of Forest Biomaterials, North Carolina State University, Raleigh, NC, United States
b Escuela de Ingeniería Química, Universidad de Los Andes, Mérida, Venezuela
c Bio-based Colloids and Materials (BiCMat) group, Department of Forest Products Technology, School of Chemical Technology, Aalto University, Espoo, Finland
d Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC, United States

Abstract

Cellulose nanofibrils (CNF) were incorporated in water-in-oil (W/O) microemulsions and emulsions, as well as water-in-oil-in-water (W/O/W) multiple emulsions using soybean oil. The addition of CNF to the aqueous phase expanded the composition range to obtain W/O/W emulsions. CNF also increased the viscosity of the continuous phase and reduced the drop size both of which increased the stability and effective viscosity of the emulsions. The effects of oil type and polarity on the properties of the W/O/W emulsions were tested with limonene and octane, which compared to soybean oil produced a smaller emulsion drop size, and thus a higher emulsion viscosity. Overall, CNF are a feasible alternative to conventional polysaccharides as stability enhancers for normal and multiple emulsions that exhibit strong shear thinning behavior.

1. Introduction

Polysaccharides such as xanthan and guar gums have traditionally been used to enhance emulsion stability [1–3]. Such effect, related to the reduction of coalescence phenomena in oil-in-water (O/W) emulsions, is ascribed to the increased viscosity of the continuous phase and to the creation of a polymer network that limits drop collisions [4]. Addition of stability enhancers is very
critical for the life time of the final emulsion, especially in food formulations. Products like mayonnaise or salad dressing contain polysaccharides to improve their shelf life without deterioration [5–7]. Cellulose is abundant, renewable, biodegradable and biocompatible [8] and can be used in preparation of emulsions for the food, pharma, cosmetic and other industries. Several cellulose grades have been utilized as a component in dispersions and emulsions. For instance, microcrystalline cellulose has for long been used in industry as a stabilizer and as a fat substitute for food products [9] as well as a texture modifier in cosmetics (skin products, etc.) and foods (ice creams, pastes, etc.) [10]. Microcrystalline cellulose is prepared via acid treatment of wood fibers, which dis-solves the amorphous regions yielding crystalline particles of low aspect ratio and with sizes in the order of tens of micrometers [11,12]. In contrast, cellulose nanofibrils (CNF) are assemblies of cellulose polymer chains and residual cell wall elements forming nanoparticles with a very large aspect ratio (fibrils) that contain both crystalline and amorphous domains. This latter cellulose form, CNF, is the subject of this investigation that demonstrates its role as an effective enhancer of the stability of emulsions. CNF is commonly prepared via mechanical disintegration of wood fibers, leading to fibril diameter as small as few nanometers and several micrometers in length [13]. They have been extensively suggested as strength promoters [14–17] and rheology modifiers [18]. CNF is also considered as an excellent shape anisotropic nano-material that is able to stabilize surfactant-free emulsions, also known as Pickering emulsions, via particle assembling and network formation at the interface [19,45].

Recently, CNF have been reported as effective stabilizer of high internal phase emulsion [20]. Due to the hydrophobic nature of cellulose and the corresponding three point contact angle of CNF at the oil/water interface, bending favors positive curvature toward the organic phase and results in water continuous, O/W emulsions [21]. Utilization of CNF in the manufacture of water-in-oil (W/O) emulsions requires CNF hydrophobization [22]. Most relevant to the present work, is consideration of nanocellulose for the stabilization of emulsions and as replacement of typical hydrosoluble polymers such as xanthan or guar gums. Thus, we present a surfac-tant system that enables ultralow energy emulsification of soybean oil in water-continuous emulsions using CNF to dramatically enhance their colloidal stability.

2. Experimental section

2.1. Materials

The cellulose nanofibrils, CNF, were produced via mechanical disintegration of a dispersion of fully bleached softwood fibers. The fibers were disintegrated by using a microfluidizer (Microfluidics M-110V) that processed the aqueous dispersions at 1.5 wt.% solid content and 20 passes. Soybean oil (MP Biomedicals), limonene (Fluka) and octane (reagent grade 98%, Sigma–Aldrich) were used as oil phases in the formulation of the emulsions. The surfac-tants, sorbitan (20) monoester (brand name Span 80, Sigma–Aldrich), polyethylene glycol tert-octylphenyl ether (brand name Triton X-100, Sigma–Aldrich) and n-pentanol (Acros Organics) were used to prepare the surfactant–oil–water (SOW) systems that generated the emulsions. Reverse osmosis water was used in all the emulsions.

2.2. Pseudo-ternary diagrams

The phase behavior of the SOW systems was monitored by using standard protocols that also allowed the construction of pseudo-ternary diagrams following the titration method [23,24]. In short, the oil phase mixed with the surfactant systems was titrated using an aqueous dispersion of CNF at given solids content. The number and type of phases in the SOW system were identified and located in a compositional pseudo-ternary diagram. Thermodynamic stability was considered as condition for the construction of the pseudo-ternary diagrams but kinetically stable phases were also identified for completeness.

2.3. Emulsion preparation and stability map

In order to evaluate the stability of the emulsions three water-to-soybean oil ratios (WOR) were considered: 25/75, 50/50 and 75/25 (reported by weight). Four different CNF concentrations in the aqueous phase were used in combination with the given WOR, namely, 0.5, 1, 1.5 and 3 wt.% (based on the mass of the aqueous phase). Limonene and octane were used to prepare 50/50 WOR emulsions with 1 wt.% CNF aqueous dispersions. A mixture of a lipophilic and a hydrophilic surfactants was also added in order to facilitate the emulsification process; specifically, this system adsorbs with a high density at the interface, leading to a reduction in interfacial tension [25]. Addition of n-pentanol as a co-surfactant further enhanced packing of the surface-active molecules at the interface and prevented gel formation. The ratio of the two surfactants and the alco-hol in the mixture was 1:1:1 by weight. The concentration of the surfactant mixture was kept constant in all systems at 3 wt.% (based on the total mass). The phase behavior of SOW mixtures and emulsions was followed at 25 °C. The emulsification protocol included first the addition of the surfactant mixture to the oil phase followed by incorporation of the CNF aqueous dispersion. Water was added as required to attain the respective WOR and the system was subjected to gentle shaking by hand for 1 min for emulsification. The resultant emulsions were evaluated for their stability during at least two weeks. Creaming or separation of excess phase in the emulsion was also recorded by direct observation.

2.4. Fluorescence imaging and emulsion rheology

Emulsions were imaged using a confocal fluorescence microscope (Zeiss LSM 710) attached to a Zeiss Axio Observer Z1. The objective used was LD C-Apochromat 40×/1.1 NA Water Immersion (Zeiss). The oil and aqueous phases were dyed using Nile Red (99% pure, Acros Organics) and Nile Blue A (Sigma), respectively. The CNF were identified with Calcofluor White Stain (Fluka). Excitation and emission used for Nile blue were 633 nm and 637–721 nm, for Nile Red 488 nm and 539–641 nm and for Calcofluor white 405 nm and 415–505 nm. The rheological behavior of the emulsions was investigated using an AR-2000 rheometer from TA instruments operating with parallel plates with a gap of 1 mm. All the rheological measurements were performed at 25 °C.

3. Results and discussion

3.1. Phase behavior of S(surfactant)–O(oil)–W(water), SOW, systems

The phase behavior of SOW systems (surfactant mixture, soy-bean oil and CNF aqueous dispersion) was recorded in pseudo-ter-inary diagrams (Fig. 1). Three distinctive SOW system zones were identified: water-in-oil microemulsion (ME), water-in-oil normal emulsion (W/O) and water-in-oil-in-water multiple emulsion (W/O/W). In general, it was observed that the concentration of CNF in the aqueous phase affected the number of zones (W/O, ME, W/O/W) present in the SOW systems and the respective range of concentrations defining the particular zone. The SOW system containing no CNF in the aqueous phase can be observed in the pseudo-ternary diagram shown in Fig. 1a. Only two
zones were found for this system (ME and W/O). An optically clear water-in-oil (W/O) microemulsion was obtained when the concentration of the aqueous phase was lower than 35 wt.%. As the concentration of water was increased above 35 wt.%, the microemulsion became cloudy and turned into a regular W/O emulsion. Addition of water above 50–60 wt.% caused a breakdown of the emulsion and rapid phase separation.

For the system containing 0.5 wt.% CNF (Fig. 1a) all the three zones were present (ME, W/O and W/O/W). The ME zone was observed at surfactant concentrations larger than 20 wt.% and water content lower than 35–40 wt.%. The composition within the ME zone in Fig. 1a represents the conditions where the interfacial tension was reduced to ultralow values and allowed spontaneous emulsification, resulting in a thermodynamically stable microemulsion with CNF located in the dispersed drops. Titration of the system containing 0.5 wt.% CNF indicated that as the concentration of such aqueous phase increased, it was no longer possible to keep it dispersed and the microemulsion (ME) transitioned to a normal type of emulsion. In this region, kinetically stable W/O emulsions were observed and located in the pseudo-ternary diagram as a region between the microemulsion (ME) and multiple emulsion (W/O/W) zones. These W/O emulsions were stable for at least 2 weeks with no evidence of creaming. The zone corresponding to W/O/W multiple emulsions was found at concentrations of the aqueous phase larger than 40 wt.% (case of CNF content of 0.5 wt.%). Emulsion inversion (from oil-continuous to water-continuous) takes place if the respective compositional line is crossed from the W/O to the W/O/W zone, as indicated in the ternary diagrams.

It is interesting to note that W/O/W emulsions were formed only if the aqueous phase contained CNF. Thus, in the formation of the multiple emulsions CNF likely play a similar role as that observed for certain polymers used in SOW systems for the same purpose [26]. In these cases, the polymer additive influences the equilibration of the surfactant at the interfaces [26], leading to a partitioning between the water and oil that favors the formation of droplets inside the larger ones (double or multiple emulsions). More specifically, the occurrence of multiple emulsions has been associated with a conflict between the composition (water-to-oil ratio) and the formulation of the system (hydrophilic-lipophilic balance of the surfactant, etc.) [27]. In the present investigation, the surfactant mixture favored the occurrence of a emulsion that is oil-continuous (W/O region in Fig. 1a) provided CNF were not present in the aqueous phase. However, the presence of CNF strongly favors the formation of water-continuous emulsions. Therefore, when the aqueous dispersion of CNF is added to the system a conflict exists between composition (favoring W/O emulsions) and formulation (favoring O/W emulsions), which translates in the appearance of a multiple W/O/W emulsion. If such addition is carried out by mixing the components in a continuous fashion, a catastrophic inversion of the initially oil-continuous phase would take place. CNF dispersed in water tends to form a network held by nanofiber entanglement. Such phenomenon together with the high viscosity of the aqueous phase favors water

Fig. 1. Pseudo-ternary phase diagrams of SOW systems consisting of a surfactant mixture (S), soybean oil (O) and water (W) containing CNF. The different diagrams were constructed from SOW composition scans with systems that contained CNF in the aqueous phase at different concentrations: 0 (a), 0.5 (b), 1.5 (c) and 3.0 (d) wt%. The “ME” regions indicate thermodynamically stable water-in-oil microemulsions. The “W/O” region represents kinetically stable water-in-oil emulsions and the “W/O/W” region represents the kinetically stable water-in-oil-in-water multiple emulsions (see also photos illustrating the visual appearance of these systems).
as the continuous phase of the system (W/O/W emulsions), i.e., when the concentration of the aqueous phase in the SOW system is higher than 40–45 wt.% (Fig. 1a). The W/O/W multiple emulsions are formed by applying low energy emulsification, for example, by gentle hand-shaking. These emulsions did not present any phase separation nor creaming within a period of 2 weeks of observation.

The role of hydrophilic biopolymers on the enhanced stability of water-continuous phase emulsions has been widely addressed [1–3]. This can be illustrated by using the modified Stokes equation (Eq. (1), [28]) to better understand the stabilizing effect of CNF if one factor the viscosity of the continuous phase ($\eta_1$), which is inversely related to the droplet (dispersed phase) velocity ($v$) under the effect of the gravitational field ($g$):

$$V = \frac{\phi_m \cdot d_f^2 \cdot (\rho_2 - \rho_1) \cdot g}{18 \eta_1} \times (1 - \phi_1)^{4.65}$$

(1)

The parameters in Eq. (1) include the maximum packing volume fraction ($\phi_m$); the effective characteristic diameter ($d_f$); the density of the oil, dispersed phase ($\rho_2$); the density of the aqueous continuous phase ($\rho_1$) and the effective volume fraction ($\phi_1$). The increased viscosity of the continuous phase (W in W/O/W) and the reduction of the terminal velocity of the (W/O) drops lead to a stable system. Such improved stability of emulsions by utilization of a highly viscous, continuous phase has been noted in the case of polysaccharides such as xanthan, arabic and guar gums [1–3,28].

The types of systems (or zones) present in the pseudo-ternary diagram as well as the respective composition domain or location depended on the concentration of CNF in the aqueous phase. The size of the W/O zone remained unchanged but that of the W/O/W region increased considerably as the CNF concentration in the aqueous phase was increased from 0.5 to 1.5 wt.% (Fig. 1a and b). This is possibly due to nanofiber entanglement that resulted in an increased viscosity of the continuous phase. These combined effects made the emulsions more stable and thus expanded the range of compositions at which W/O/W multiple emulsions were obtained.

When the concentration of CNF in the aqueous phase was further increased to 3.0 wt.% only the W/O and W/O/W zones were observed, that is, the ME region was reduced or disappeared. This can be explained by the formation of a strong CNF gel, which was challenging to disperse and hence limited the conditions for the typical spontaneous emulsification of microemulsions. The increased size of the W/O/W zone is also probably related to the conditions of increased viscosity of the continuous phase.

The W/O zone was observed at surfactant concentrations higher than 60 wt.% and aqueous phase content below 20 wt.%. For aqueous phase content higher than 20 wt.% multiple W/O/W emulsions were always formed. The formation of multiple W/O/W emulsions is the result of the presence of CNF, especially at high concentrations, which limits the dispersion of water droplets in oil-continuous emulsions (W/O). Therefore, a conflict between factors that favors oil-continuous emulsions (including formulation and the WOR or water to oil ratio) and the high viscosity of the aqueous phase containing CNF leads to a compromised system, i.e., multiple emulsions (W/O droplets dispersed in W continuous phase). Finally, we note that the drop sizes were ~200 nm, similar in magnitude to the characteristic length of the CNF.

### 3.2. Formulation, composition and stability of the emulsions

The phase behavior of the SOW systems as indicated by the pseudo-ternary diagrams was used as a basis for the formulation of kinetically stable systems. Different W/O/W emulsions were prepared by utilizing the conditions determined in Fig. 1, changing the water-to-oil ratio (WOR) and the concentration of CNF in the aqueous phase. The effect of these parameters on the stability of the emulsions was investigated. As such a stability diagram was constructed at 25 ºC (Fig. 2) for systems with a fixed surfactant concentration (3.0 wt.% and water content from 10 to 90 wt.%). Three different regions were observed: (1) an unstable region where the components of the emulsion rapidly separated as soon as agitation was ceased; (2) a stable W/O/W region where a kinetically-stable multiple emulsions were formed by gentle agitation and, (3) a creaming region where the obtained emulsions creamed after a period of time of less than two weeks.

When the concentration of water was kept below 35 wt.%, the conditions for the formation of W/O/W emulsions were limited to CNF content in the aqueous phase below 0.5 wt.% (Fig. 2). When the concentration of CNF was increased up to 3.0 wt.%, the system became unstable and the formation of a stable emulsion with water as the continuous phase was not possible; instead, the components of the emulsion rapidly separated into the original components, soon after agitation was ceased. The possible reason for this behavior is that the high concentration of oil promotes aggregation of CNF (and thus phase separation) due to the difference in polarity. The high CNF hydropathy favors its location in the continuous aqueous phase, away from the interface with oil. When the fraction of oil was high enough and the concentration of CNF was 1 wt.% or more, the exclusion of CNF from the inter-drop space was dominant and induced both drop collapse and CNF agglomeration. However, when the concentration of CNF was small enough, below 0.5 wt.% for example, the fibril network remained well dispersed in the aqueous phase and favored a stable emulsion.

For water content in SOW systems between 25 and 75 wt.%, and irrespective of the CNF content in the aqueous phase, multiple W/O/W emulsions were formed upon gentle agitation (1 min agitation). This is likely due to the viscosifying effect of CNF. A similar behavior has been observed for other polysaccharides that are able to increase the viscosity of the aqueous phase and reduce emulsion creaming [1–3].

When the concentration of water in SOW systems was increased over 65 wt.% and the concentration of CNF was below 1.0 wt.%, the obtained emulsions creamed in a period of time of
less than two weeks. However, such creaming was not observed when the concentration of CNF was above 1.0 wt.%. Thus, when the fraction of water was over 65 wt.% the efficiency of the surfactant mixture decreased, probably due to a dilution effect (relative large water volume). Formation of a stable system would require a very high aqueous phase viscosity, which CNF is not able to provide if used at concentrations below 1 wt.%.

3.3. Emulsion morphology

W/O/W multiple emulsions were prepared based on the identified conditions for stable emulsions and imaged by using confocal fluorescence microscopy (Fig. 1 and 2). The images in Fig. 3a–d correspond to WOR = 1 emulsions with CNF contents in the aqueous phase ranging from 0.5 wt.% (Fig. 3a) to 3.0 wt.% (Fig. 3d). It is apparent that as the concentration of CNF in the aqueous phase was increased, the drop size of the W/O/W emulsion decreased. The drop size reduction is caused by the increased viscosity of the continuous phase (CNF effect). The same effect has been observed for xanthan gum and other polysaccharides that are known to be effective at reducing the emulsion drop size and improving emulsion stability by the increased viscosity of the continuous phase [29–31]. The cellulose fibrils were located in the continuous phase (as can be observed by the fluorescence trace in Fig. 3e), creating a network that contained the oil droplets. This effect can be also observed in Fig. 3f where the oil and the CNF are distinguished by the red and green colors assigned to the respective dyes. The CNF network in the continuous phase of the emulsion clearly has two effects: First, it immobilizes oil droplets, reduces collisions and prevents drop coalescence and growth. Secondly, the network facilitates improved adsorption of the surfactant at the interface due to the extra time provided by the reduction of the terminal velocity of the drops when they are held in the polysaccharide network. In other words, when an emulsion is prepared, the creation of interface is required and the success of emulsion formation is linked to the ability of the surfactant to reach the interface before the collapse of the drops [32]. As it was explained before, the presence of CNF in the aqueous phase reduces the velocity of the dispersed drops, and allows time for the surfactant to diffuse from the bulk, reach and adsorb at the interface, as shown by other authors who have used polysaccharides in emulsion systems [1–3,32,33].

An interesting feature of the emulsions observed in Fig. 3a–d is their morphology, which as explained before, consisted of multiple emulsions with the aqueous phase dispersed inside oil droplets in an external aqueous phase. The water phase was dyed and imaged (Fig. 3g) to confirm that it was indeed the external phase and also the dispersed phase inside the oil droplets of the emulsion. The occurrence of these multiple emulsions can be facilitated by the mixture of hydrophilic and lipophilic surfactants, which enables stabilization of water-in-oil in the drops as well as their dispersion in water [34,35]. However, this is the first time that such emulsions are reported for CNFs. Multiple emulsions have typically been used for encapsulation of food and drug additives [35,36] and the use of CNF in such system can open some new alternative uses.

3.4. Rheological characterization of the emulsions

A rheological characterization of the emulsions was performed in order to better understand the stabilization mechanism. The flow curves for WOR = 1 and concentrations of CNF in the aqueous phase varying from 0.5 to 3.0 wt.% reveal that as the concentration of CNF increases the apparent viscosity of the resulting emulsion also increases (Fig. 4a). This phenomenon can be explained by the increased viscosity of the aqueous dispersion containing CNF and related entanglement [37,38]. Although the viscosity of the continuous phase could be the most important factor contributing to emulsion viscosity, the drop size is also an important factor. As it
was shown in Fig. 3, the drop size was reduced from ca. 16 to ca. 5 μm when the concentration of the CNF in the aqueous phase was increased from 1.0 to 3.0 wt.%. The reduced drop size increases the emulsion interfacial area and friction that results in a higher viscosity [39,40].

The flow curves of two emulsions with water as the continuous phase, with the same CNF concentration but different content of the internal phase are presented in Fig. 4b. It can be observed that as the WOR is changed from 1 to 0.33 (25/75, W/O), the viscosity of the emulsion is slightly increased. This behavior has been found for other systems [39] and is explained by an increased interfacial area in high internal phase emulsions and related inter-drop friction.

The rheological data were adjusted to the Herschel–Bulkley model in order to quantify the changes in the rheological behavior of the emulsions as a function of CNF concentration in the aqueous phase (Eq. (2)):

\[
\tau = \tau_0 + \gamma^n m
\]

where \(\tau\) represents the shear stress, \(\tau_0\) is the yield stress for flow, \(m\) is the consistency index; \(\gamma\) is the shear rate and \(n\) is the flow index (see Table 1 for the best fits).

It can be observed that regardless of the CNF concentration, all the emulsions present a shear-thinning behavior. As the concentration of CNF is increased, the yield stress of the emulsion also increases. Likewise, the consistency index increases with the addition of CNF to the aqueous phase in the emulsion. The more significant increment in yield stress and consistency index occurred when CNF concentration increased from 1.5 to 3.0 wt.%, which correlates with the significant change in the viscosity of the emulsions. This result is expected since as the concentration of CNF increases, the connected fibril network leads to gelation of the continuous phase and increases the force required for the system to flow. These results are in agreement with other results obtained for emulsions containing xanthan gums [41]. Such emulsions have a yield stress that is higher than the buoyance-driven stresses of the drops (dispersed phase), which prevents the creaming of the emulsion. As an example, an emulsion of soybean oil–in–water with a characteristic drop size of 10 μm, the value for the buoyance-driven stress is around 8 mPa, which can be compared to the yield stress determined for CNF-containing emulsions investigated here, which is at least 1000-fold higher. This fact highlights the important role of CNF as stability enhancer.

### 3.5. Influence of oil type on the morphology and rheological behavior of CNF stabilized emulsions

Emulsions were prepared with limonene and octane as the oil phase in order to identify the effect of the oil type on the characteristics of the emulsions. These emulsions were prepared using similar conditions to those for soybean oil emulsions (WOR = 1 and surfactant concentration of 3.0 wt.%, CNF content of 1.0 wt.%). The drop size of the emulsion prepared using soybean oil was ca. 11 μm (Fig. 5a), which was larger than that of the emulsions prepared with limonene (ca. 2 μm) and octane (ca. 4 μm) (Fig. 5b and c). These differences in drop size are most likely related to the properties and characteristics of the oil phases used, for instance molecular structure and interfacial tension against water. There are various factors that play a role in the evolution of the characteristic drop size of an emulsion, namely, coalescence, flocculation and Ostwald ripening [42]. However, the drop size measured when different oils were used indicate a predominant effect of the nature of the oil (molecular structure). Soybean oil is composed of a mixture of fatty acids with long carbon chains, with an equivalent alkane carbon number (EACN) of 18 (the EACN is the number of carbon atoms that a linear alkane would have to result in the same phase behavior) [43]. Limonene is a terpene with fewer carbon atoms than the fatty acids in soybean oil and has an EACN close to 8 [23]. Octane is a linear alkane with only 8 carbon atoms (EACN = 8). It appears that the surfactant mixture used in the present work is more effective for emulsification of oils with an EACN of 8 and produce emulsions with smaller drops. Another observation from Fig. 5 is that the emulsions with limonene and octane are not in fact multiple emulsions, as was the case with the soybean oil. This effect is likely to be caused by the structural differences of the oils used.

We note that emulsions prepared with the same composition as presented in Fig. 5 but without CNF in the aqueous phase separated

### Table 1

<table>
<thead>
<tr>
<th>CNF wt.%</th>
<th>(\tau_0) (Pa)</th>
<th>(m) (Pa s^n)</th>
<th>(n)</th>
</tr>
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<tbody>
<tr>
<td>0.5</td>
<td>1.5</td>
<td>0.7</td>
<td>0.58</td>
</tr>
<tr>
<td>1.0</td>
<td>2.3</td>
<td>1.4</td>
<td>0.54</td>
</tr>
<tr>
<td>1.5</td>
<td>3.7</td>
<td>7.4</td>
<td>0.44</td>
</tr>
<tr>
<td>3.0</td>
<td>19.5</td>
<td>14.8</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Fig. 4. Flow curves of WOR = 1 emulsions with varying concentrations of CNF in the aqueous phase, as indicated (a). Results for emulsions with WOR = 0.33 (25/75) as well as WOR = 1 as reference are also included in (b) for a CNF concentration in the aqueous phase of 0.5 wt.%.
(creamed) much faster, after about 20 min. This confirms that the presence of CNF in limonene and octane emulsions improved their stability, as was also the case for soybean oil.

The flow curves for the emulsions prepared with limonene and octane, as well as soybean oil are presented in Fig. 5d. Important differences in the apparent viscosity, mainly due to differences in drop size, can be noted at low shear rates, with the emulsion prepared with limonene presenting the highest viscosity. A small drop size or high interfacial area leads to large interfacial friction and produce a high viscosity. As the shear rate is increased (above 10 1/s), the apparent viscosity of the emulsions becomes similar.

4. Conclusions

The use of cellulose nanofibrils (CNF) facilitates the preparation of multiple emulsions consisting of W/O droplets dispersed in an external, aqueous phase (W/O/W emulsions). We demonstrate the use of CNF as emulsion stability enhancer, which adds to its application as emulsifier [20]. In contrast to processes involving multiple steps [44], the procedure introduced here for the first time enables the formation of multiple emulsions in a single step. Construction of pseudo-ternary diagrams for the systems containing surfactant mixture, soybean oil and aqueous CNF revealed that at low CNF concentration it is possible to form a microemulsion with oil as the continuous phase but as the concentration of CNF in the aqueous phase is increased to 3.0 wt.% the system becomes a kinetically stable W/O emulsion. For low aqueous phase content the emulsions are unstable but at 25 wt.% or higher they become stable. The stability effect is enhanced with an increase in CNF concentration. A possible reason for these observations is the formation of a swollen fibrillar gel network that prevents droplet collision and thus improves emulsion stability, which is in agreement with the emulsion stabilizing effect of other polysaccharides [1–3]. As the CNF concentration increases, the formation of a more connected network takes place, resulting in higher viscosity and smaller drops. The flow curves of the emulsions were found to adjust well to the Herschel-Bulkley model and indicated yield stresses and consistency indices that increased with CNF concentration. The drop size and viscosity of the emulsions were found to be dependent on the type of oil used. Overall, this work shows that CNF is effective for dramatically enhancing emulsions stability and can be used as replacement to conventional polysaccharides. The choice of oil and formulation variables allows the design of normal or multiple emulsions stabilized by CNF.

5. Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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