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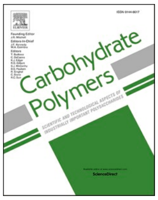

On the formation and stability of cellulose-based emulsions in alkaline systems: Effect of the solvent quality

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ABSTRACT

With amphiphilic properties, cellulose molecules are expected to adsorb at the O/W interface and be capable of stabilizing emulsions. The effect of solvent quality on the formation and stability of cellulose-based O/W emulsions was evaluated in different alkaline systems: NaOH, NaOH-urea and tetrabutylammonium hydroxide (TBAH). The optimal solvency conditions for cellulose adsorption at the O/W interface were found for the alkaline solvent with an intermediate polarity (NaOH-urea), which is in line with the favorable conditions for adsorption of an amphiphilic polymer. A very good solvency (in TBAH) and the interfacial activity of the cation lead to lack of stability because of low cellulose adsorption. However, to achieve long-term stability and prevent oil separation in NaOH-urea systems, further reduction in cellulose's solvency was needed, which was achieved by a change in the pH of the emulsions, inducing the regeneration of cellulose at the surface of the oil droplets (in-situ regeneration).

1. Introduction

Emulsions have a wide-spread interest and their uses range from technical to life science applications (Walstra, 2005). During the emulsification process, different stabilizing and emulsifying agents have been used. Among them, surfactants, synthetic and natural macromolecules, and particles are often used (Kronberg et al., 2014b). Synthetic surfactants and polymers have played an important role, but for reasons of sustainability and safety there is a strong trend towards alternatives based on natural and renewable resources. Here both lipids and other macromolecules should be highlighted. Carbohydrate-based systems include novel surfactants and systems based on cellulose. In this respect, cellulose derivatives have a long tradition; both nonionic, anionic and cationic derivatives find a broad use in emulsion formulations, such as foods, pharmaceuticals, personal care products and water-based paints (Murray, 2009; Sarkar, 1984; Wüstenberg, 2014). Cellulose nanoparticles, first described and made on purpose by Bengt Rånby (1951), have rather recently been introduced as emulsion stabilizers (Kalashnikova et al., 2011). Their action resembles the typical Pickering effect (Pickering, 1907).

The preparation and stabilization of emulsions relies on different mechanisms depending on the emulsifier used. Low molecular weight molecules, such as surfactants and lipids, have an important role in decreasing the interfacial tension between the oil and water phases thus reducing the energy needed for dispersion. On the other hand, macromolecules have a different main role; by adsorbing at the oil-water interface, they stabilize the emulsions by creating a “steric” barrier. The repulsion depends on the extension of the polymer chains away from the interface and thus a favorable interaction between the polymer and the continuous medium; when droplets approach each other, a repulsion arises both due to reduced solvation and due to a decreased conformational entropy of the extending polymer chains (Kronberg et al., 2014b). Amphiphilic polymers and nonionic surfactants, such as those with long ethylene oxide chains, can act as both emulsifiers and

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stabilizers.

Except for molecular stabilizers, it has been known for a long time that small particles may stabilize dispersions in general, and emulsions in particular. As mentioned above, such emulsions are known as Pickering emulsions after the pioneer in the field (Pickering, 1907). Cellulose nanoparticles belong to this class of stabilizers (Salas et al., 2014). For a good stabilization, the particles should primarily be wetted by the liquid making up the continuous medium; the contact angle is an important factor judging the efficiency of particle stabilization (Dickinson, 2010). It is believed that the amphiphilic character of cellulose nanoparticles resides in its crystalline organization at the elementary brick level, and thus, since cellulose nanocrystals have both hydrophilic and hydrophobic edges, these will be preferentially wetted by water or oil, respectively (Kalashnikova et al., 2012). In recent years there has been a development of the so-called Janus particles (Kumar et al., 2013), i.e., particles with an amphiphilic character that are composed of two or more regions with distinct physicochemical properties; these can provide efficient stabilization. Whereas cellulose derivatives are well established as emulsion stabilizers and various nanocelluloses have been receiving an increasing interest, we believe that molecularly dissolved cellulose should be also considered as an efficient natural candidate for emulsion formation and stabilization (Costa, Medronho, et al., 2019).

Cellulose dissolution has been an active field for a long time but in recent years there has been a strongly renewed interest, not only driven by UN's agenda for a sustainable development, but also as a result of new emerging applications of cellulose. Many solvents have been developed throughout the years and are well described in literature, ranging from ionic liquids and organic systems to water-based ones (Medronho & Lindman, 2014). With the goal of using pristine cellulose for emulsion preparation and stabilization, the most direct way would consist in having unmodified cellulose soluble in one of the constituent liquids since, otherwise, solvent removal would require additional laborious steps in the manufacture. Cellulose is not soluble in water or oil but can be dissolved in certain aqueous systems at high or low pH's; this can be ascribed to an ionization effect by protonation or deprotonation giving cellulose a polyelectrolyte-like character (Bialik et al., 2016; Lindman et al., 2010; Lindman et al., 2017). In addition, it has been observed that the dissolution can be facilitated by amphiphilic additives or by using acids or bases with organic counterions (Alves et al., 2015; Alves et al., 2016b; Medronho & Lindman, 2014, 2015). The reason for this is expected to have a marked contribution from cellulose's amphiphilic character and concomitant role of hydrophobic interactions. Therefore, reducing the hydrophobic interactions by having acids or bases with “hydrophobic” ions or adding substances known to reduce hydrophobic interactions, are important aspects to consider when developing novel solvent systems and rationalizing cellulose's dissolution mechanism. Urea is a common example of such an additive. Its weakening effect on hydrophobic interactions does not only significantly reduce the tendency of surfactant self-assembly or affect protein and DNA stability, but also enhances cellulose dissolution (Lindman et al., 2021).

Although cellulose dissolution in aqueous media is a central issue for emulsions, some aspects related to its dissolution and self-assembly mechanisms are still debatable (Lindman et al., 2021). Nevertheless, its clear amphiphilic features are expected to drive cellulose to self-assemble at oil-water interfaces and be able to stabilize emulsions. As mentioned, recent studies have found that cellulose nanocrystals display amphiphilic properties and can give stable emulsions. It was further observed that nanocrystals with low surface charge favor the stability of emulsions, suggesting that the amphiphilic nature of cellulose is the main driving force for the stabilization (Kalashnikova et al., 2012).

Molecularly dissolved cellulose is likely to behave closer to typical cellulose derivatives, namely the cellulose ethers, widely used commercially as emulsion stabilizers (Hon, 2001; Jedvert & Heinze, 2017; Murray, 2009; Seddiqi et al., 2021). However, native cellulose has been much less explored due to the mentioned dissolution limitations. In our previous works, we have been focusing on acidic water-based solvents for cellulose molecular dissolution and emulsion formation (Costa et al., 2021; Costa, Medronho, et al., 2019; Costa, Mira, et al., 2019; Medronho et al., 2018). We have shown that molecular cellulose dissolved in aqueous solutions of phosphoric acid (H_3PO_4 (aq.)) adsorbs at the oil-water interface (Costa, Mira, et al., 2019). For instance, the interfacial tension between oil and H_3PO_4 (aq.) solution used to dissolve cellulose was found to be lowered by the presence of cellulose and this decrease was similar in magnitude to that displayed by nonionic cellulose derivatives in water (Wüstenberg, 2014). From these results, it can also be rationalized that the amphiphilicity of cellulose derivatives is, to a large extent, due to the hydrophobic cellulose backbone (Lindman et al., 2021). Furthermore, the aging of the cellulose-stabilized emulsions formed in that same solvent was evaluated; emulsions were found to be short-lived, as oil was separating from the emulsions and floating to the top within 24 h. However, by adding water to the dispersions as a second step during emulsification, the properties of the emulsions changed dramatically, and there was no evidence of oil separation over one year of storage (Costa, Mira, et al., 2019). This effect was attributed to a decrease in cellulose solvency with water, thus acting as an “antisolvent”. The decreased solvency promotes a greater affinity for the oil-water interface, which, in turn, may lead to the remarkable emulsion stability against macroscopic phase separation.

With the present work we wanted to extend the knowledge on the emulsification capabilities of dissolved cellulose to another type of aqueous non-derivatizing solvents, the alkaline, and moreover, understand how the solvent quality (i.e., cellulose dissolution state) may affect the migration of cellulose molecules to the O/W interface, and thus, emulsion stabilization. The selected alkaline (aqueous-based) solvents were NaOH (Alves et al., 2015; Alves et al., 2016a), NaOH-urea (Alves et al., 2016a; Cai & Zhang, 2005) and TBAH (Alves et al., 2015; Alves et al., 2016a). Recent research suggests that in the two latter cases, cellulose dissolves down to the molecular level whereas in neat NaOH (aq.) cellulose aggregates persist (Alves et al., 2015; Alves et al., 2016a). Different methods were used to infer on the effect of the solvent system properties on emulsion formation and stability. These included tensiometry, turbidimetry, optical microscopy, laser diffraction, microrheology and carbohydrate quantification.

2. Materials and methods

2.1. Materials

The cellulose source was microcrystalline cellulose (Avicel PH101, Sigma Aldrich) with a weight-average molecular weight (M_w) of ≈62,000 g/mol and a polydispersity index of 3.85, as determined by size exclusion chromatography. NaOH (99.2% purity), dodecane (100%), hydrochloric acid (HCl-37%) and sulphuric acid (H_2SO_4–95%) were purchased from VWR chemicals. Urea (≥99% purity), TBAH (40 wt% aqueous solution), α-D-glucose (anhydrous, 96%), orcinol (97%) and calcofluor white stain, were obtained from Sigma Aldrich. MilliQ water was used in the preparation of all aqueous solutions. Sulfate-latex particles (8 w/v% aqueous solution) were purchased from ThermoFisher Scientific.

2.2. Cellulose dissolution

Solutions of 0.5 wt% cellulose were prepared with three different alkaline aqueous solvents: 7 wt% NaOH (1.9 M), 7 wt% NaOH-4 wt% urea (1.9–0.75 M) and 40 wt% TBAH (1.5 M). The solutions were prepared following an adapted procedure (Pereira et al., 2018). Briefly, the cellulose powder was added to the alkaline solvents at room temperature (20–21.5 °C) and all samples were vigorously stirred for 1 min with a vortex. The sample with TBAH was kept under mild stirring at room temperature overnight, until the cellulose was completely dissolved. The samples with NaOH and NaOH-urea were placed in the freezer at −30 °C for 10 min, removed and then vigorously stirred (while cold) for another minute. This way, the suspensions become more homogeneously mixed and stable until the complete freezing of the samples. The solutions were kept in the freezer overnight and then thawed at room temperature. Clear solutions were obtained after this procedure and dissolution was confirmed by polarized light microscopy.

2.3. *Cellulose emulsions and suspensions*

O/W emulsions were prepared by dispersing 10 wt% dodecane in the different cellulose solutions for 5 min using an Ultra-Turrax mixer at the speed of 16,000 rpm. For a total mass of 25 g emulsion, small beakers of 40 ml and a diameter of 4 cm were used to perform the homogenization of all different alkaline systems. Subsequently, emulsions were prepared for each solvent system by decreasing the pH of the continuous phase by the addition of precise amounts of HCl solution (1–3 ml, i.e., 0.012–0.036 mol of acid per addition). This procedure allows to follow the changes occurring in emulsion formation and stability upon the progressive decrease of cellulose solubility, for each solvent system. The highest amount of acid added (0.036 mol) was observed to decrease the pH of the NaOH-based emulsions below 13.5, which is the pK_a value reported for cyclodextrins (a good cellulose model candidate) (Gaidamauskas et al., 2009). Below this pH, cellulose is expected to have a lower charge density. For TBAH, even though used with a lower molarity than NaOH, the pH was still higher than 13.5, which might be a result of its superior basicity. For these emulsions post-regenerated with acid, a second step in the emulsification was considered; for each ml of HCl solution added, 3 extra min of stirring with the Ultra-Turrax were performed. The HCl solution was added drop-wise while emulsions were stirring at 16000 rpm, to ensure a fast spreading of the acid to the whole system and to avoid, as much as possible, heterogeneous cellulose regeneration. Following this procedure, no major clumps of regenerated cellulose were visually observed. Control samples of the different regenerated systems were made following the same procedure as previously described, but without the addition of the oil. This resulted in simple suspensions of regenerated cellulose in the different alkaline solvents.

2.4. *Turbidimetry of cellulose suspensions*

The turbidity of the regenerated suspensions of cellulose in the different alkaline solvents was determined using a Hach RATIO/XR 43900 turbidimeter, equipped with a tungsten lamp. The turbidity readings were performed directly on the instrument, using a nephelometric turbidity unit (NTU) scale, based on white light (400–680 nm) and 90° incident angle. The measurements were performed with matched Hach glass sample cuvettes with screw caps at room temperature (20–21.5 °C) and performed immediately after preparation.

2.5. *Diffusion wave spectroscopy of cellulose emulsions*

A RheoLab instrument (LS Instruments AG, Fribourg, Switzerland), equipped with the echo technology, was used for diffusing wave spectroscopy (DWS) analysis in the transmission mode. DWS is an advanced light-scattering technique that accesses the microrheology of highly turbid samples by approximating the propagation of light to the diffusion equation; a detailed theoretical framework of this method can be found elsewhere (Niederquell et al., 2012; Reufer et al., 2014). Briefly, an emulsion contains oil droplets that perform Brownian motions, which are sensitive to the rheology of the system. When a laser source illuminates the sample loaded in a cuvette, the photons will be scattered multiple times by the oil droplets. Intensity fluctuations are detected when the scattered photons emerge at the opposite side of the incident light. These intensity fluctuations are then used as a probe to characterize the rheological properties of the sample. A glass cuvette made of 5 mm in thickness (L), was used and all the samples were vigorously mixed for 120 s, using a vortex mixer, and immediately measured at 25 °C. The measurement time was set to 120 s with additional 60 s of echo duration. Three individual runs for each sample were performed, and the estimated error was below 5%. Before measurements, the l^* calibration (being l^* the transport mean free path in the medium) was performed using as reference standard an aqueous solution composed of 0.55 wt% of sulfate-latex particles of mean diameter of 500 nm, which guaranteed a deviation of less than 20% between the CR of the sample and the standard. The calibration was performed using the Rheolab l^* calibration mode at 25 °C and a glass cuvette of the same thickness as used for the samples.

2.6. *Optical microscopy of cellulose suspensions and emulsions*

Samples were observed on a Leica DMRX light microscope. Typically, a small droplet of the dopes was placed on a glass slide and covered with a cover slip. Samples were analyzed by transmitted light polarization using crossed polarizers, at magnifications of 10× and 20×. Pictures were captured using a Leica DFC 320 camera with 5 megapixels. Samples were also stained with calcofluor white and observed in an Axioimager Z2 (Carl Zeiss) fluorescent microscope and recorded with the b/w camera Axiocam HRm (Carl Zeiss). Images were acquired and processed using the Axiovision 4.8 software (Carl Zeiss) and ImageJ (open source software from the National Institutes of Health, USA).

2.7. *Droplet size measurements of cellulose emulsions*

The median droplet diameter (D50) of the cellulose-based emulsions was determined by laser diffraction using a Mastersizer Hydro 2000SM instrument, using a He-Ne gas laser red light with a beam wavelength of 633 nm and a LED blue light with a wavelength of 466 nm (Malvern Instruments, UK). For these measurements, a small volume of the emulsions was added drop-wise into a sample dispersion unit (at 2000 rpm), filled with water, until an optimum measuring value was reached by the program (i.e., an indirect indication of the number of drops).

2.8. *Interfacial tension of cellulose solutions/dodecane*

The force tensiometer K20 (Krüss) equipped with a Wilhelmy plate, was used to measure the interfacial tension (IFT) between the different cellulose-alkali solutions and dodecane. Cleaning of the plate and beakers was done by flushing the materials with acetone, ethanol and water, in that exact order, and then the materials were dried and placed in a plasma cleaner for 3 min. This procedure was repeated before each measurement, followed by the plate calibration, which was performed by measuring the surface tension between air and water (values between ca. 72.5–73 mN/m were typically obtained). Measurements were taken every 5 min during a period of 60 min for each sample.

2.9. *Carbohydrate content in the emulsion serums*

The carbohydrate content of the serums of the post-regenerated NaOH-based emulsions was determined using the Orcinol method, following the SCA-F W 15:77 protocol (SCA R&D Centre, 2011). The Orcinol method is based on a color reaction of carbohydrates with 0.2% orcinol reagent dissolved in concentrated sulphuric acid. A calibration curve was obtained by preparing calibration solutions of D-glucose with 0.1 g accuracy, and the concentration of carbohydrates in the sample of interest was then calculated from the equation of this calibration curve. The absorption of the samples was measured using a UV-1800 Shimadzu UV–vis spectrophotometer at a wavelength of 480 nm.

3. Results and discussion

3.1. *Effect of solvent quality on the adsorption of cellulose at the oil-water interface*

An important goal of this work was to better understand the mechanisms behind the adsorption and interfacial activity of cellulose molecules at the oil-water interface, and to determine how the solvent quality affects such behavior. This was done by using alkaline water-based solvents with different features, such as polarity and amphiphilicity, in order to perceive what are the most suitable dissolution conditions for cellulose, which successfully contribute to stabilize O/W emulsions. The solubility of cellulose is increased in the following order: NaOH < NaOH-urea < TBAH (Alves et al., 2016a). The interfacial activity of cellulose was assessed by measuring the IFT between the oil-phase (dodecane) and the different solvents containing cellulose (Fig. 1A). In the NaOH-based solvents, the interfacial tension decreases with the presence of cellulose, with the decrease being more pronounced in the solvent with an intermediate polarity (NaOH-urea). On the other hand, with the TBAH solvent it is more intriguing to characterize the interfacial behavior of cellulose, since the solvent itself gives a quite low IFT due to the presence of the amphiphilic cation TBA^+; in fact, cellulose seems to affect the IFT slightly in the opposite way, which may be ascribed to a competition between the TBA^+ ions and cellulose for the interface.

In discussing the interfacial behavior of cellulose, the complications due to adsorption of solvent constituents at the interface must be accounted for. An amphiphilic ion like TBA^+ is expected to have a significant adsorption at oil-water interfaces as it is clearly confirmed by our observations (Tamaki, 1967). This makes inferences on cellulose adsorption from such solutions difficult; the slight increase in IFT suggests, however, competition with TBA^+ for the interface and some cellulose adsorption. Urea has been found to give a weak decrease of the IFT in the related decane-water system (Jones, 1973). In our present work, no major differences were noted between the NaOH and NaOH-urea system (Fig. 1A, grey curve "NaOH-based solvents"). Therefore, the downward shift of the IFT curve from the NaOH to the NaOH-urea system may be ascribed to the enhanced dissolution of cellulose in the latter case, where more individual cellulose molecules may be available to adsorb at the interface more efficiently. To conclude about the interfacial behavior of cellulose from the lowering of the IFT, it is clear from the results for the two NaOH solvents that cellulose shows a significant adsorption at the oil-water interface; this is in line with the results for acidic solvents and also in line with an amphiphilic character of cellulose. For the TBAH case, the amphiphilic properties of the solvent cation preclude clear conclusions from IFT measurements; direct measurements like ellipsometry or neutron reflection could be helpful. In lack of clear information, it can be noted that in an environment of amphiphilic ions like TBA^+ we do not expect a major driving force for cellulose adsorption.

3.2. *Emulsification ability of cellulose as a polymeric emulsifier*

The study of the IFT gives a basis for investigations of the ability of

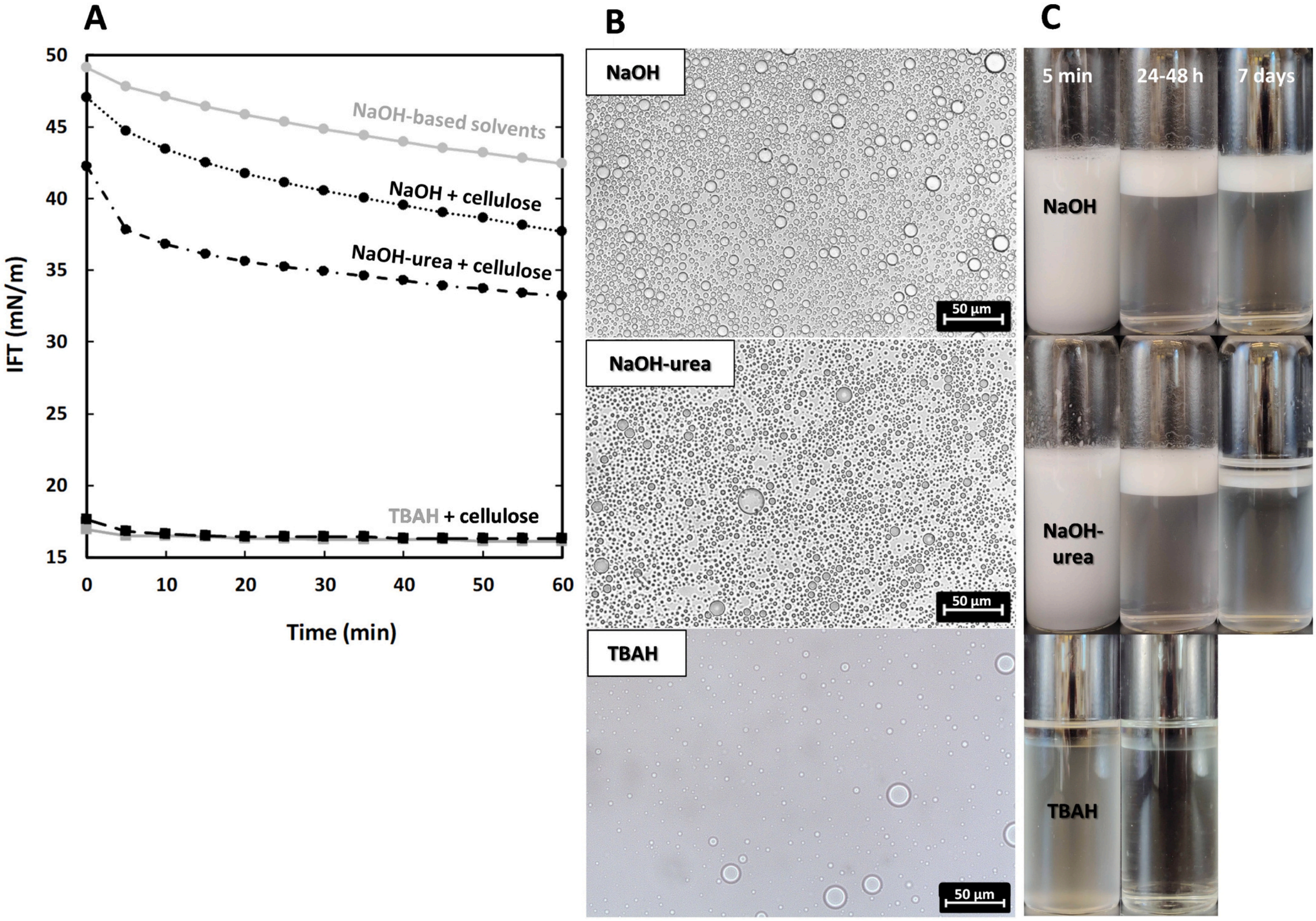


Fig. 1. A) Interfacial tension between dodecane and the alkaline solvents (grey curves) and respective cellulose solutions (black curves); the grey dotted curve "NaOH-based solvents" represents both alkali solvents: NaOH and NaOH-urea. B) Light microscopy micrographs of the resultant emulsions stabilized by cellulose in the NaOH-based solvents. C) Visual appearance of the vials; stability against oil separation in the different alkali-based emulsions.

cellulose to adsorb at oil droplets in O/W emulsions and thus to stabilize the emulsions. O/W emulsions were prepared with the different alkaline solvents, and their behavior was first followed (visually) with time. As it is observable from the photos in Fig. 1C for the TBAH system, already 5 min after homogenization the oil was almost completely separated from the emulsion, with only a few droplets being left in the emulsion; these droplets were separating in 24 to 48 h. In the case of NaOH-based solvents, visual oil separation was only noticed after one week. This phenomenon was, however, more pronounced in the solvent system composed of NaOH-urea (Fig. 1C).

Stabilization of dispersions by polymers can be discussed mainly from two effects; the degree of polymer adsorption and the conformation of the polymer chains at the surface (Kronberg et al., 2014a). A discussion can be fruitfully based on solvency effects. A poor solvency leads to poor dispersion stability since the polymer layer will have limited thickness. On the other hand, a very good solvency leads to lack of stability because of low adsorption. Therefore, optimal stabilization is attained at intermediate solvency conditions (if the polymer has an amphiphilic character this is favorable for adsorption and stability). These arguments are based on the adsorption of individual polymer molecules from solution. If the polymer is not dissolved to the molecular level but is aggregated, a more complex behavior can be expected including the presence of polymer aggregates or "lumps" on the surface; then additional stabilization can be achieved due to the presence of relatively thick polymer layers.

The emulsions morphology (Fig. 1B) and droplets median sizes (D_{50}) were obtained by light microscopy and laser diffraction, respectively. The droplet sizes were only measured for the NaOH-based systems. Because both NaOH-based emulsions had a bimodal distribution, volume and number of distributions were both considered for the calculation of D_{50}. Approximate values were obtained for both emulsion systems, 18–20 μm in volume and 3–5 μm in number, with spans of 1.4 to 3 (see also the size distributions in Fig. S1). Looking at the micrographs in Fig. 1B, small micrometer droplets in the approximate size as given by the number distribution, can be actually observed which seems to be in good agreement.

3.3. *Effect of solvent quality on cellulose regeneration at the oil-water interface*

In acidic aqueous solvents, emulsions stabilized by cellulose molecules are short-lived, but adding water in a second step of the emulsification process induces cellulose regeneration at the interface, and provides outstanding long-term stability (one year or even more) (Costa, Medronho, et al., 2019; Costa, Mira, et al., 2019). In the present study, an alternative approach was followed: cellulose solubility was progressively decreased by decreasing the solution pH as second step of the emulsification procedure. The changes occurring in the emulsions upon pH decrease were followed by visual inspection. In Fig. 2, the first vial for each solvent system represents the neat emulsions without any acid addition, i.e., the emulsions stabilized by cellulose adsorbed at the O/W interface. Upon the progressive in-situ regeneration, data strongly suggests that the adsorption of cellulose at the interface is affected by the solvent quality. In the NaOH-based systems, the picture is similar, but there are a few differences worth noting. For both cases, the stability against oil separation increases, and opposite to what happened before regeneration for the NaOH-urea system, now the stability against oil separation matches the one for the NaOH system. This can be explained by a change in the physical state of the cellulose molecules and the formation of a soft-hard cellulose "shell", a more robust mechanical barrier to prevent oil escaping. It is also noticed that the stability against creaming improves upon further pH decrease. This means that more cellulose is being regenerated and taking part in stabilizing the oil droplets, either by adsorbing to the interface or contributing to the regenerated cellulose network in the continuous phase. Both emulsion systems present clear serums (depleted of oil droplets) at the bottom of the vials, which indicates that cellulose steric stabilization is not strong enough at the concentration used for this study (i.e., 0.5 wt%). However, the serum of the NaOH-urea system has a smaller volume compared to the NaOH system, indicating an improvement in creaming stability. In the NaOH-urea system, on the second vial (first acid addition) it is noteworthy that part of the regenerated cellulose seems to be settling after a while (i.e., 24–48 h), but it cleared out after shaking and aging again. This cellulose sedimentation is probably due to a weak network of cellulose that starts regenerating in the bulk, and existing mostly in the form of flocks due to the low concentration used. This is sometimes also seen in the NaOH-based system, but less evident than for NaOH-urea solvent.

The case of TBAH is strikingly different from the NaOH-based solvents. The first acid addition does not seem to have an effect on cellulose adsorption to the interface; for the second acid addition there seems to be a small layer of oil on top, with an emulsion in the middle and a clear serum at the bottom. Further analysis by visual inspection with time and the fluorescence microscopy reveals that oil droplets are actually "trapped" in a viscous network of regenerated cellulose, which eventually will separate and part of the regenerated cellulose will sediment covering the serum part. This means that, when dissolved in TBAH, cellulose regenerates in the bulk rather than at the interface of the emulsions. To get a better insight into the differences between regenerating cellulose in the different alkali systems and also to compare the regeneration of cellulose in the bulk versus in the presence of oil droplets, cellulose suspensions were analyzed by turbidimetry, and later, both suspensions and emulsions were analyzed by fluorescence microscopy.

In Fig. 3, it can be observed that the suspensions of both NaOH-based solvents are not visually very different, but the turbidity measurements tell otherwise. For the highest amount of acid added, a substantial decrease in turbidity for the suspensions in NaOH-urea is observed,

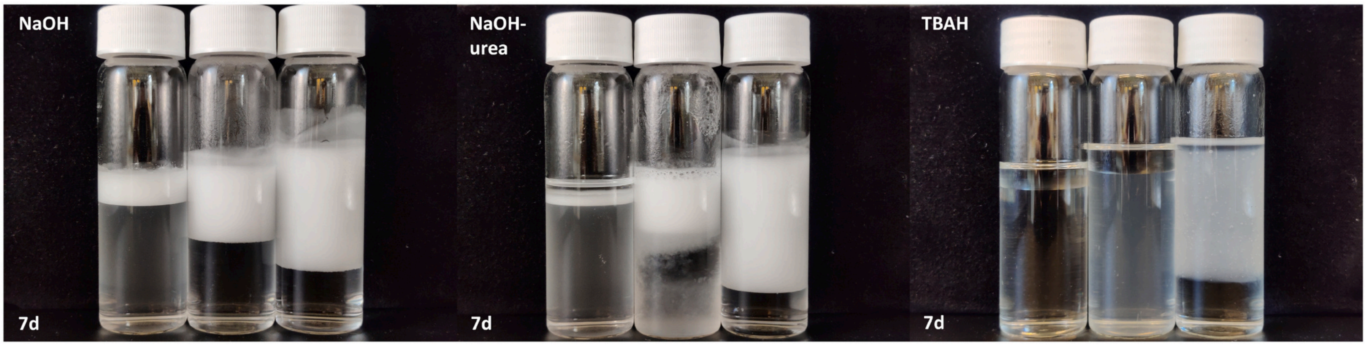


Fig. 2. Photographs of short-time stability tests (7 days) of post-regenerated emulsions. For each solvent system, equal moles of acid were added progressively from the left to the right vials. The first vial in each solvent system represents the emulsion without acid (only dissolved cellulose).

which can be translated into a different organization of the cellulose molecules upon regeneration from a solvent with an intermediate polarity, and possibly more amorphous structure, as it has been shown by Alves et al. (2016a). It is also possible to see that after 48 h of aging of the suspensions, the stability against sedimentation increases in the order: NaOH < NaOH-urea < TBAH (Fig. 3). This observation could possibly relate to the slightly better stability against creaming of the post-regenerated emulsions in the NaOH-urea solvent (3rd vial on Fig. 2) given that, from droplet size measurements, no significant differences are detected for both NaOH-based systems (i.e., D_{50} was calculated to be 16–17 μm from the volume distributions, and 6 μm from the number distributions, with spans of 1.2 to 1.6; see also the size distributions in Fig. S1). It was also observed, that below a certain pH (slightly below the pKa value), the sizes of the emulsions are no longer affected by pH and cellulose is expected to be more or less irreversibly adsorbed at the emulsion interface. For the TBAH system, it is noteworthy that precipitation of the cellulose molecules is only observed upon the addition of three times more acid than for the NaOH-based systems. This can be reasoned by the higher basicity of the TBAH, which possibly leads to a higher charge density of the cellulose chains. Consequently, a higher amount of acid is needed to neutralize enough charges and trigger cellulose regeneration (Bialik et al., 2016).

When comparing the regeneration of cellulose in TBAH systems in the presence or absence of oil, no apparent difference is seen (Fig. 4, 3rd column). Fluorescence microscopy revealed a similar structure of the regenerated cellulose network with no oil droplets stabilized by cellulose, since no fluorescence is detected around the droplets seen in the picture (the fluorescence arises from the bulk). On the other hand, for both NaOH systems (Fig. 4, 1st and 2nd columns), the oil droplets reveal a thin fluorescent layer of cellulose surrounding them and a network of cellulose in the continuous phase. Therefore, we can conclude that cellulose molecules stabilize emulsions in a very similar way as its cellulose derivatives do: a) by reducing the interfacial tension, arising from the amphiphilic character of the cellulose backbone; b) adsorption of a cellulose layer that provides steric repulsion; and c) formation of a network on the continuous phase.

3.4. *Carbohydrate content in the serums of the emulsions*

In our previous work, it was found that the stabilized acidic emulsions exhibited creaming after 24 h of storage, leaving a clear liquid fraction "serum" (depleted of droplets) at the bottom. This was attributed to the aggregation of droplets due to the neutralization of the cellulose charges when dissolved at extreme pH conditions (Costa, Medronho, et al., 2019; Costa, Mira, et al., 2019). Similar aggregation and creaming phenomena of the emulsions are expected to occur in the alkaline-based aqueous solvents. If cellulose is diffusing from bulk solution to the emulsion interface upon pH decrease, then the carbohydrate content in continuous phase (serum) is expected to decrease. In Table 1 it is possible to observe that the progressive addition of HCl makes the continuum media depleted from cellulose. On the other hand, as the degree of dissolution is enhanced by the presence of urea, it is not surprising that a higher cellulose concentration is still found in the serum of the NaOH-urea system in comparison to the neat NaOH solvent. Since cellulose is expected to be more aggregated in the NaOH system than in NaOH-urea, larger particles are expected to adsorb at the emulsions interface in the former solvent while molecularly dissolved cellulose (or close to it) will adsorb in the latter case, thus leaving more cellulose available in the bulk. When the pH decreases substantially, the solvent quality becomes poorer and the majority of cellulose is expected to be found at the emulsions interface, regardless the NaOH system. These results clearly demonstrate that the dissolution degree of cellulose together with the solvent quality (here affected by the pH) are fundamental parameters to understand the emulsion formation and stability in these strongly alkaline systems. Note that no determination of carbohydrate content was performed in the TBAH system due to the poor stability of the emulsions formed.

3.5. *Microrheology and stability of emulsions*

The rheological characterization of the emulsions is of major importance since it provides information on their physical stability and on the molecular features of the continuous phase. Therefore, the microrheological properties of the regenerated NaOH-based emulsions systems were extracted by DWS transmission measurements; the complex viscosity (η*), storage (G′) and loss (G″) moduli are displayed in Fig. 5a and b.

The first observations are that the emulsion systems have similar η*. This agrees with the rather similar average size observed in both NaOH-systems and similar content of sugars in the continuous phase (see Table 1). Since the crossover occurs at high frequencies, we can assume that the samples are essentially viscous. From this crossover, the

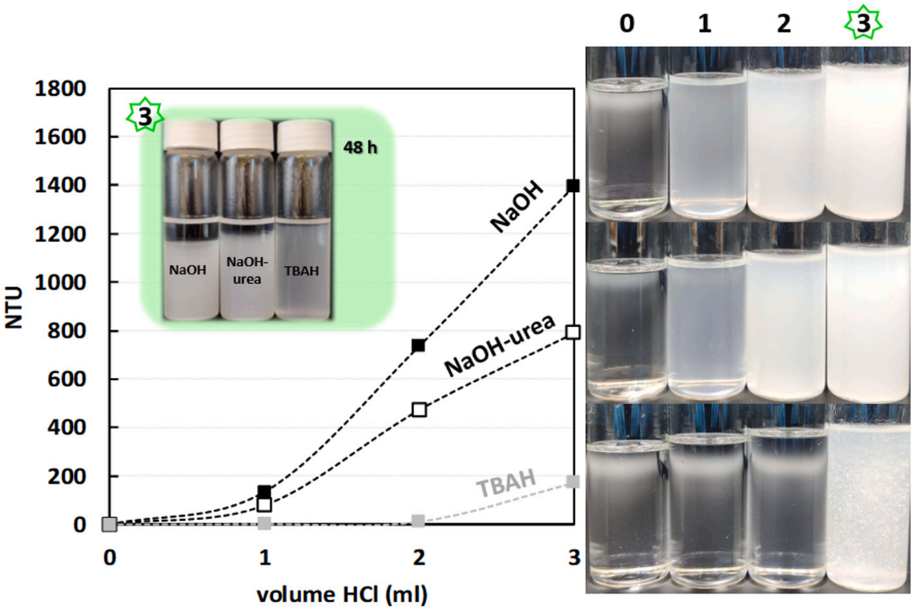


Fig. 3. Turbidity of the cellulose suspensions in the different alkaline solvents in freshly prepared samples; images of the vials correspond to the respective solvent in the curves, i.e., NaOH, NaOH-urea and TBAH, from the top to the bottom. The numbers on top of the vials correspond to the HCl (37 wt%) volume additions. The samples with 3 ml acid addition were aged for 48 h and the results are shown in the green square. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

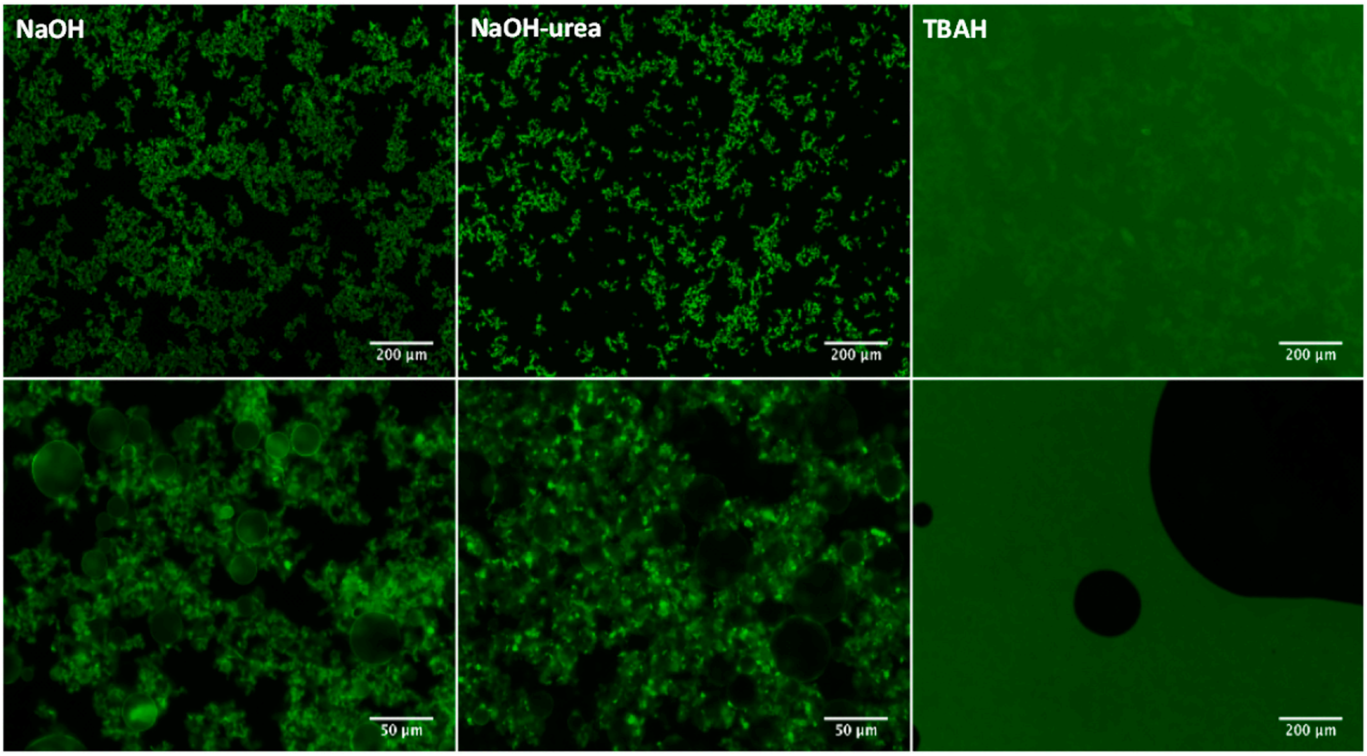
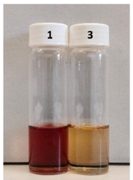


Fig. 4. Fluorescence micrographs of the cellulose suspensions (on top) and emulsions (on the bottom) of each alkali system. All samples were stained with calcofluor white. (3 ml HCl solution addition for both emulsions and suspensions).

Table 1

Carbohydrate content estimated in the continuous medium (serum) after acid addition by the Orcinol method (SCA R&D Centre, 2011). Images show the colorations acquired by the serums upon reaction with the orcinol reagent; lighter colors mean a reduction in carbohydrate content.

Initial cellulose concentration (g/l)	Volume HCl added (ml)	Final cellulose concentration (g/l)	
		Emulsion serum NaOH	Emulsion serum NaOH-urea
5.8	1	2.5 ± 0.23	3.2 ± 0.04
5.8	3	0.4 ± 0.06	0.3 ± 0.00



relaxation time, τ, was estimated as ca. 8.83×10^{-5} s and 4.62×10^{-5} s for NaOH and NaOH-urea systems, respectively. As discussed, urea is known to improve cellulose dissolution; the decrease in this short-time relaxation with the addition of urea in the solvent system can suggest that the degree of cellulose dissolution may affect the dynamics and relaxation mechanisms that occur at the microscale (high frequencies), such as dissipation mechanisms.

DWS analysis performed in the transmission mode also enables to infer about the emulsions stability by evaluating the intensity correlation function (ICF) decay over the aging time of the emulsion. The ICF decay consists on the normalized temporal fluctuations of the scattered light intensity and is sensitive to the concentration and size of the tracers (i.e., oil droplets). For larger and/or less concentrated oil droplets, the ICF decay will occur at longer lag times. On the other hand, a decay to short lag times is expected for more concentrated droplets and/or smaller and comparatively rapid ones. Consequently, a shift of the ICF decay to long lag times, over the aging time of the emulsions, can reflect the onset of destabilization mechanisms, such as Ostwald ripening, creaming, flocculation or coalescence (Medronho et al., 2018). Therefore, the stability of the regenerated NaOH-based emulsions with time was followed by DWS in the transmission mode during 48 h and the differences in the normalized ICF vs. lag time during sample aging were evaluated (Fig. 6a and b).

A shift towards higher lag times in the ICF decay was obtained for both samples tested. The moment when the ICF equals 1 corresponds to the moment at which no scattered light is detected, indicating the roughly complete physical separation between the oil and water phases, and it occurred after 30 min for the NaOH sample and 60 min for the NaOH-urea. To better compare the onset of destabilization, the $t_{2/3}$ parameter, corresponding to the lag time at which the ICF curve decays to 2/3 of its initial value, was determined for both samples and plotted as a function of the aging time (Fig. 7a).

The onset of destabilization occurs at approximately the same time for both samples, since the $t_{2/3}$ parameter starts to increase after ca. 27 min for the NaOH emulsion and ca. 30 min for the NaOH-urea emulsion. The enhanced dissolution of cellulose in the NaOH-urea system allows for a dissolution state closer to the molecular level, presumably making cellulose molecules capable of diffusing and adsorbing more efficiently at the oil-water interface, thus enhancing its stability and delaying the complete phase separation.

The transport mean free path, l^*, corresponds to the average distance a photon needs to travel before completely losing its direction (randomization). It is sensitive to the concentration of the scattering particles present in the solution and, for this reason, it can also contribute to elucidate the mechanisms involved in the emulsion destabilization processes. The l^* parameter of a given sample (l^*_{sample}) is also related to its count rate (CR_{sample}), through the equation (Medronho et al., 2018):

$$l^*_{sample} = l^*_{ref} \times \frac{CR_{sample}}{CR_{ref}}$$

where the CR is defined as the average measure of the photons arriving in the detector; CR_{ref} and l^*_{ref} are estimated by measuring a calibration standard and the CR_{sample} is obtained during the ICF measurement of the

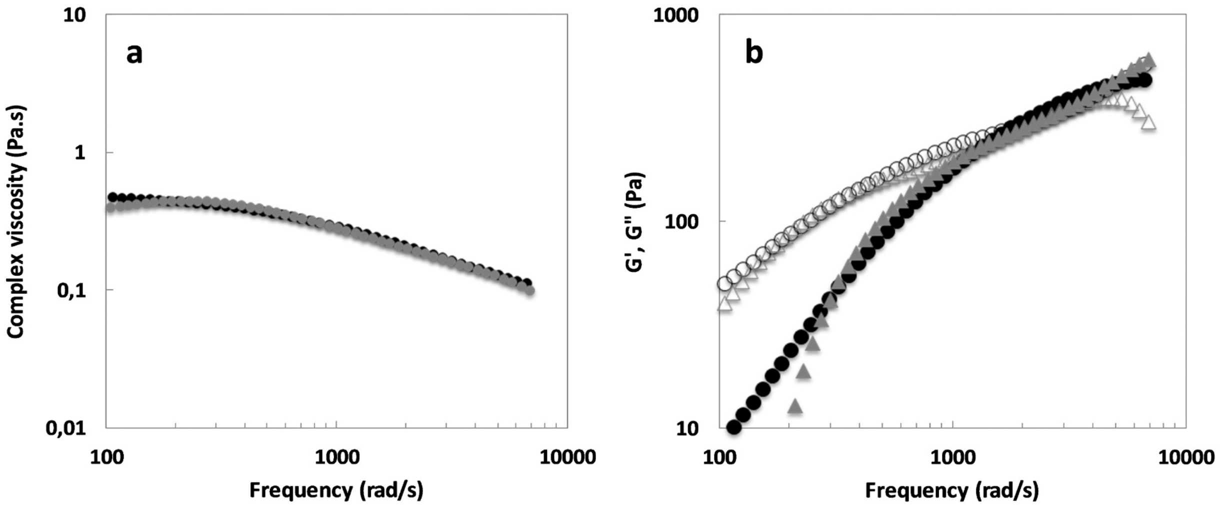


Fig. 5. Viscoelastic parameters determined by DWS at 25 °C of the regenerated NaOH (grey circles) and NaOH-urea (black circles) emulsion systems: (a) complex viscosity (left); (b) G′ (full symbols) and G″ (empty symbols).

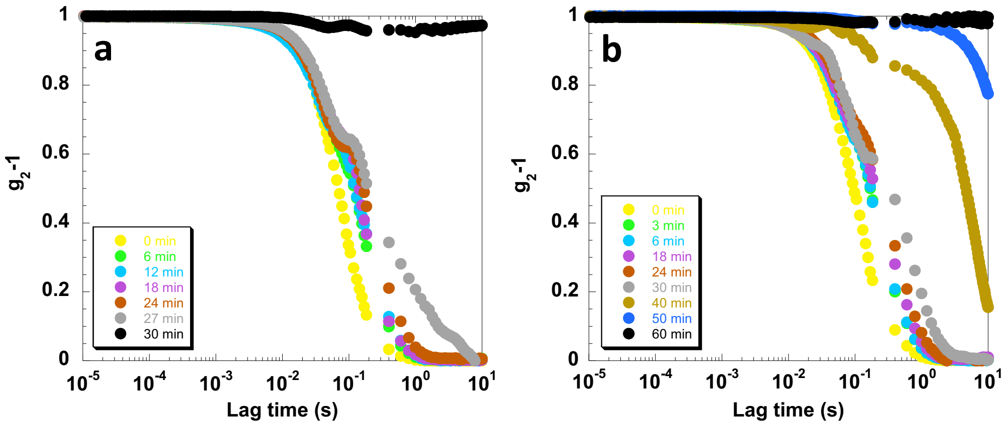


Fig. 6. Normalized ICF curves obtained for the NaOH (a) and NaOH-urea emulsions (b) at different times.

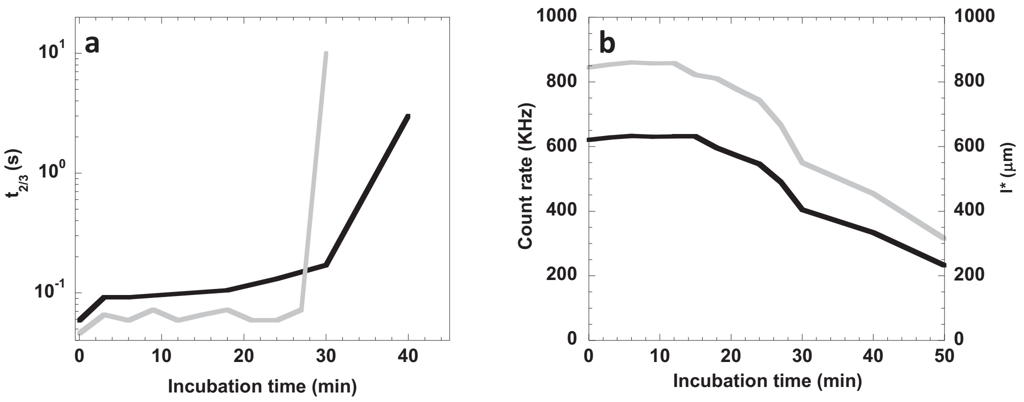


Fig. 7. (a) $t_{2/3}$ Parameter for the NaOH (grey line) and NaOH-urea (black line) emulsions over time. (b) Evolution of the count rate (black line) and l* (grey line) parameters for the NaOH-urea sample.

sample. As can be observed in Fig. 7b, a gradual decrease over time of both parameters is obtained for the NaOH-urea system. Similar profile was observed for the neat NaOH solvent (data not shown). These results suggest that the decrease in the *l** parameter might be due to a decrease in the number of scattering events over time. This further indicates that the emulsion's destabilization might result from creaming, since the migration of the oil particles to the upper layer of the solution during the aging would result in a decrease of the scattering events, leading to a decrease in *l** and *CR*. In our previous work with stabilized acidic emulsions (Costa et al., 2021; Medronho et al., 2018), the *l** and the $t_{2/3}$ parameters exhibited a similar trend but increasing with the aging time. This similar trend might reflect the occurrence of the Ostwald ripening phenomenon since, at a constant volume fraction of oil, there is a concomitant increase of the particles size with a decrease in their number density, leading to larger values of l^*. Nevertheless, we expect to further elucidate on the destabilization mechanisms of cellulose stabilized emulsions in forthcoming works.

4. Conclusions

The formation and stability of cellulose-based O/W emulsions in different alkaline systems were evaluated focusing on the effect of solvent quality. Cellulose was found to decrease the IFT substantially and this effect is ascribed to the cellulose migration to the oil-water interface. In this respect, the TBAH (aq.) was found to induce the lowest IFT itself without a significant contribution from cellulose. It is suggested that this effect is due to the amphiphilic behavior of the TBA^+ cation and its capacity to compete with cellulose for the oil-water interface. The practical consequence of this result is that when oil is added and emulsions formed, their stability is very poor (due to the lack of cellulose at the interface) as demonstrated by fluorescence microscopy and aging tests. On the other hand, the addition of urea to NaOH (aq.) is expected to improve cellulose dissolution and therefore more cellulose in the form of molecules will be available to adsorb at the emulsion interface (as confirmed by the carbohydrate quantification), stabilizing it more efficiently than the neat NaOH system. These results found support from the microrheology assays where, in addition, the destabilization mechanism of the emulsions formed in the NaOH systems is suggested to occur via creaming. In conclusion, the adsorption behavior of cellulose is dependent on the solvent quality. Intermediate solvency conditions are favorable for cellulose adsorption at the interface, which is in line with the typical behavior of amphiphilic polymers. A very good solvency of cellulose (in TBAH) leads to a lack of stability due to low adsorption of the molecules, and oil will quickly separate from the emulsion. Intermediate solvencies allow cellulose to stabilize emulsions in a short-term. To achieve long-term stability, further solvency decrease is needed after oil dispersion in the cellulose solution, what we call the in-situ regeneration. The rate of droplets coalescence is then dramatically reduced and emulsions show a remarkable stability against oil-separation.

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CRediT authorship contribution statement

Carolina Costa: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Bruno Medronho:** Conceptualization, Writing – review & editing, Supervision. **Alexandra Filipe:** Investigation, Writing – review & editing. **Anabela Romano:** Writing – review & editing, Supervision. **Björn Lindman:** Conceptualization, Writing – review & editing. **Håkan Edlund:** Writing – review & editing, Supervision. **Magnus Norgren:** Conceptualization, Writing – review & editing, Supervision.

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.

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