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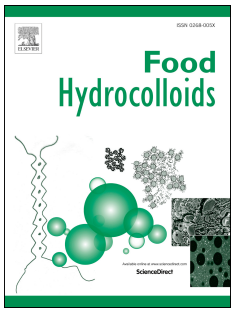

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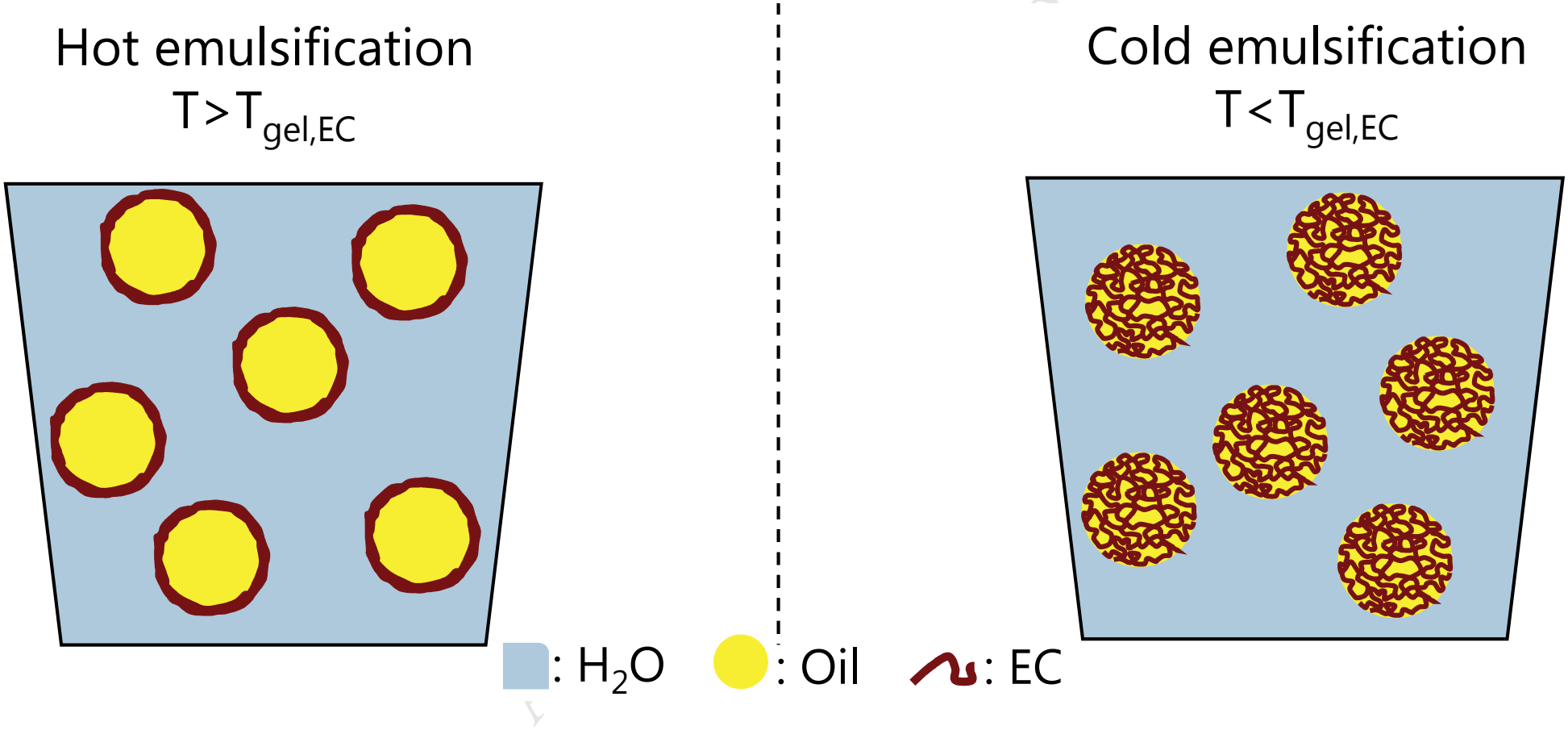

Oleogelating properties of ethylcellulose in oil-in-water emulsions: The impact of emulsification methods studied by ^{13}C MAS NMR, surface tension and micropipette manipulation studies

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Abstract

This study addressed the oleogelating properties of EC when EC-oleogel microdroplets are dispersed in an aqueous medium. By measuring the interfacial tension between oil-water, EC was found to be interfacial active. Oleogel-in-water emulsions were prepared by two different emulsification methods termed *hot* and *cold*. The first included high pressure homogenization of EC-oil and water at a temperature above the gelling point of EC, whereas the latter implied dispersion of set EC-oleogels in water by high speed mixing at a temperature below the melting point of EC-oleogels. The oleogelling functionality was lost when hot emulsification was applied. Instead EC migrated to the interface of oil and water and formed a shell around oil droplets which was assessed by micropipette manipulation techniques. On the other hand, the oleogel remained stable when EC-oleogel was dispersed in water using the cold emulsification method. For this system a fraction of the triglycerides in oil was immobilized in a similar manner as oil in bulk oleogels and the mechanical properties of dispersed droplets were no longer reflecting the flow behavior of low viscous oil, which indicates oil gelation by EC.

Keywords: Ethylcellulose, oleogel, emulsion, interfacial activity, solid-state NMR, micropipette manipulation

1. Introduction

The desire to reduce the content of saturated fatty acids in the diet and the search for a more sustainable replacement of palm oil has in recent years led to increased focus on ethylcellulose (EC) as an oleogelator. EC is the only known food-grade polymer that can structure oil phases directly without applying costly

intermediate processing steps such as solvent exchange or solvent removal (Mattice & Marangoni, 2017). Liquid oil can be gelled by heating the semi-crystalline EC in the oil above the glass transition temperature, T_g, of EC and subsequently cool it below the gelling temperature, T_{gel}. T_g, T_{gel}, and the melting temperature, T_m, of EC depend on its molecular weight, which is directly correlated with the polymer viscosity and consequently EC is sold according to viscosity expressed in centipoise (cP) (Davidovich-Pinhas, Barbut, & Marangoni, 2014, 2015a) . The physical properties of EC-oleogels are affected both by compositional and processing parameters. Heating EC and oil above T_m (~180 °C) rather than T_g (~140 °C) results in oleogels of higher mechanical strength as the polymer can reorganize itself when the entire fraction of crystals are melted (Davidovich-Pinhas, Gravelle, Barbut, & Marangoni, 2015). High storage temperature of the molten gel during setting is likewise increasing the strength of oleogels (Davidovich-Pinhas, Gravelle, et al., 2015). Furthermore, a positive correlation between gel strength and enhanced polarity of the sample exists regardless of the polar components that arise from oil oxidation, addition of surfactants or type of oil (Davidovich-Pinhas, Barbut, & Marangoni, 2015b; Gravelle, Davidovich-Pinhas, Zetzl, Barbut, & Marangoni, 2016; Gravelle, Barbut, & Marangoni, 2012). The explanation for the gel strength being easily influenced by such compositional changes is that EC-oleogels are based on inter-polymer junction zones created through formation of hydrogen bonds between free unsubstituted hydroxyl groups (Laredo, Barbut, & Marangoni, 2011). At increased oil polarity additional hydrogen bonds between EC and oil are formed and consequently the gel strength is enhanced (Gravelle et al., 2016).

By formation of oleogels it is hypothetically possible to mimic and thus replace saturated fat in food products. In certain food products such as whipped cream, ice cream and baked goods the macromolecular structure is highly dependent on crystallinity of saturated fatty acids though. In whipped cream and ice cream elasticity opposing coalescence of dispersed fat globules allows formation of a three-dimensional structuring network rather than coalescence of liquid droplets and furthermore elasticity of fat in pastries provides a laminating effect between dough sheets and prevents cross-linking of gluten proteins (Baardseth, Næs, & Vogt, 1995; Goff, 1997). This emphasizes the need for not only elucidating the physical properties of bulk oleogels, but also to understand and optimize the behavior of oleogels in food product matrixes if successful substitution of saturated fat should be implemented.

For the purpose of increasing the ratio of unsaturated fatty acids and decreasing the total fat content, EC oleogels have been applied in laboratory scale to several food products such as cream cheese (Bemer, Limbaugh, Cramer, Harper, & Maleky, 2016), comminuted meat products (Zetzl, Marangoni, & Barbut, 2012), and sausages (Barbut, Wood, & Marangoni, 2016). Overall, full or partial substitution by EC oleogels was evaluated as promising ways to reduce the amount of saturated fat in these types of products.

Less focus has been directed toward EC-oleogel applications in emulsions such as whippable cream and ice cream. Recently, EC was applied to ice cream produced with sunflower oil (Munk, Munk, Gustavson, & Risbo, 2018), but the physical behavior of EC in this kind of emulsion system still needs to be clarified. As the oleogel in such systems is dispersed as microdroplets, the gel formation and properties are not straightforward to study as compared to bulk oleogels, and consequently other experimental techniques besides rheology and texture analysis have to be employed. The objective of this study is to examine the oleogelating properties of EC when EC-oleogels are dispersed in an aqueous phase. This was evaluated according to two different emulsification procedures; one executed at temperatures above the gelling point of EC (notated as hot method) whereas the other implied dispersion of the set oleogel at temperatures below the gelling point of EC (notated as cold method). The studied emulsion matrix was either based on an ice cream formulation or a simple oil-in-water model system depending on the analyses. A combination of surface tension measurements, micropipette manipulation techniques and solid-state NMR was combined to reach the objectives.

Utilization of EC to solidify liquid oil microdroplets of emulsions may open up for new possibilities to interchange saturated fats with unsaturated oils in a wide range of food products. Most food either have a high water activity or even a continuous aqueous phase in which the fat phase is dispersed. Therefore it is of vital importance to investigate the physical behavior of EC in oil being in direct contact with an aqueous phase.

2. Materials & Methods

Two grades of Ethylcellulose (EC), Ethocel Standard Premium 10 and 20, with viscosities of 10 and 20 centiPoise (cP) were provided by Dow Wolff Cellulosics, Bomlitz, Germany. Both grades of EC have a degree of substitution around 2.5, whereas the chain length of the cellulose backbone differs and thus the resulting viscosities. High oleic sunflower oil (HOSO), Fritex HOSO, was from AAK, Karlshamn, Sweden. A distilled monoglyceride with high content of glycerol monooleate (GMO), Dimodan® MO 90/D, was used as surfactant and provided by Dupont, Brabrand, Denmark. Guar gum, Grindsted Guar, and kappa carrageenan, Carrageenan 100, used as stabilizers in emulsions were also from Dupont. Sodium caseinate, Miprodan 30, and lactose were purchased from Arla, Brabrand, Denmark. Maltodextrin DE 15, C*Dry MD 01910, was from Cargill, Haubourdin, France, and sucrose from Nordic Sugar, Copenhagen, Denmark.

2.1 Preparation of EC oleogels for NMR measurements

Pure EC oleogels were prepared for NMR measurements. For the solid-state NMR analyses EC oleogels were prepared by heating 10 wt% cP10 or cP20, 3 wt% GMO and 87 wt% HOSO to 180 °C under continuous

stirring on a hotplate magnetic stirrer and holding the mixture at this temperature for additionally 10 minutes to ensure complete melting of the polymer. The molten gel was immediately transferred to 4 mm (o.d.) NMR rotors with a volume of 80 µL using glass Pasteur pipette and cooled to ambient temperature.

2.2 Preparation of EC oleogel-in-water emulsions

EC oleogel-in-water emulsions were prepared for both NMR measurements and the micropipette manipulation experiments. For the solid-state NMR analyses the composition of emulsions were tailored to ice cream formulations: with 10 wt% HOSO, 1 wt% EC, and 0.3 wt% GMO in the lipid phase and 1 wt% sodium caseinate, 12 wt% sucrose, 5 wt% lactose, 5 wt% maltodextrin, 0.15 wt% guar gum, and 0.02 wt% carrageenan in the water phase. For micropipette droplet manipulation measurements the water phase constituted just plain deionized water, as optical transparent samples are needed, **Figure 1**. Furthermore, this technique requires larger droplets in order to study individual droplets, whereas solid-state NMR analyses can be performed on realistic food emulsions containing small droplets sizes.

EC oleogel-in-water emulsions were prepared by two different methods referred to as *hot* and *cold*. For the hot preparation, the water phase was heated to 80 °C in a water bath. Simultaneously, EC (cP10 and cP20), GMO and HOSO were heated to 180 °C under continuous stirring, held at this temperature for 10 min, and cooled to 90 °C whereupon it was mixed with the hot water phase. At this point, EC-oil was not set as a gel but was still liquid. For the emulsions made for solid-state NMR analyses a heavy-duty laboratory mixer (Silverson L4RT, Silverson Machines, Bucks, UK) was used for pre-homogenization followed by a two-stage high-pressure homogenization at 150/50 bar (Panda Plus 2000, GEA Niro Soavi, Parma, Italy). A water bath connected to the heating jacket of the feed hopper maintained the temperature of 80 °C during the entire emulsification process. For the emulsions made for the micropipette droplet manipulation technique a simple emulsification was performed by mixing the hot oil/EC and hot water on a vortex mixer for approx. 15 s.

The cold preparation method included formation of an EC-oleogel and a water phase. The EC-oleogel (cP10 and cP20) was produced by heating EC, HOSO and GMO to 180 °C for 10 min and subsequently cool it to room temperature where it was allowed to set for approx. 24 h. The water phase was produced the following day as described above for the hot methods, now with the modification that the water phase was heated and subsequently cooled to room temperature before homogenization. In conclusion, cold emulsified emulsions were homogenized at room temperature, **Figure 1**. For the emulsions made for solid-state NMR analyses, homogenization was conducted with a high-speed blender (Omni-mixer homogenizer 17106, Sorvall, Newtown, CT, USA) equipped with 2 inch exterior rotor knives with an agitation speed of

16.000 rpm for 10 minutes. For the emulsions made for the micropipette droplet manipulation technique the emulsification was performed by mixing the oleogel and water phase approx. 15 s on a vortex mixer generating a wide range of droplet sizes. For the micropipette experiments, the emulsions were added to the microscope chamber so that droplets of appropriate sizes were chosen for micropipette manipulation. For NMR analyses, both emulsions from hot and cold preparation were transferred to 4 mm (o.d.) NMR rotors with a volume of 80 µL using glass Pasteur pipette.

	Hot	Cold
NMR	Liquid-liquid homogenization High-pressure T = 80 °C Ice cream formulation (water phase: sugars, stabilizers, NaCas)	Gel-liquid homogenization High speed blender T = 20 °C Ice cream formulation (water phase: sugars, stabilizers, NaCas)
MDMT	Liquid-liquid homogenization Vortex mixer T = 80 °C Simple emulsion (deoinized water)	Gel-liquid homogenization Vortex mixer T = 20 °C Simple emulsion (deoinized water)

Figure 1. Overview of homogenization methods of the emulsions prepared by the hot and the cold preparation for solid-state NMR and micropipette droplet manipulation technique (MDMT). For all emulsions, EC-oil had been undergoing thermal treatment at 180 °C, and then cooled to either 90 °C (still in liquid state: hot) or 20 °C (set gel: cold).

2.3 Solid-state ^{13}C NMR spectroscopy

^{13}C single-pulse (SP) magic angle spinning (MAS) and ^{13}C cross-polarization (CP) MAS NMR experiments were carried out at room temperature on a Bruker Avance 400 spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at Larmor frequencies of 400.13 and 100.62 MHz for ^{1}H and ^{13}C, respectively, using a double-resonance probe equipped for 4 mm (o.d.) rotors. All spectra were recorded at a temperature of 294 K and a spin-rate of 10000 Hz. For the SP/MAS experiments a recycle delay of 128 s and 300-512 scans were used, whereas a recycle delay of 8 s, 1024-6144 scans and a contact time of 1.0 ms (rf-field strength of 80 kHz for both ^{1}H and ^{13}C) were utilized for the variable amplitude CP/MAS experiments (Peersen, Wu, Kustanovich, & Smith, 1993). High-power TPPM (Bennett, Rienstra, Auger, Lakshmi, & Griffin, 1995) ^{1}H decoupling (rf-field strength: 80 kHz) was applied during an acquisition time of 49.2 ms. All spectra were referenced (externally) to the carbonyl resonance of α-glycine at 176.5 ppm.

Determination of the relative ratio of fatty acids and cellulose in the samples were obtained by integration of the spectral regions 11-22 ppm (A), 23-27 ppm (B) and 50-110 ppm (C). These regions represent the methyl groups from ethyl and the fatty acids, two specific carbons in the fatty acids (CH_2 next to methyl and –[CH_2]-CH_2-C=O), and cellulose + CH_2 from the ethyl, respectively. The molar fatty acid-to-cellulose ratio was then calculated as: 3*int(B)/(int(C)-int(A)+0.5*int(B)).

2.4 Interfacial tension

Solutions of EC (cP10 and cP20) in HOSO and sodium caseinate in MilliQ water respectively were prepared in the following concentrations: 0.03%, 0.3%, 1.0% and 3.0%. EC was melted and dissolved in HOSO by heating to 180 °C; solutions remained fluid as gelation is induced at concentrations >3%. The standard micropipette method developed by Lee et al. (2001a, 2001b) was used for surface tension measurements against air, where the micropipette was simply inserted into the microchamber, and was filled with air. For the interfacial tension measurements between water and liquid oil solutions of EC, the oil phase was loaded into the micropipette prior to insertion into the microchamber. The aqueous solution was kept inside the microchamber and was aspirated under low controlled negative suction pressure into the micropipette to form the interface of interest. Using calibrated-digital analysis, the standard way of measuring interfacial tension is by placing a measuring box as seen in **Figure 2A**.

The box in **Figure 2A** gives a measure of X and Y, which are mathematically converted to the radius of curvature, R_C, by using eq. 1.

$$R_c = \frac{\left(\frac{Y}{2}\right)^2 + X^2}{2X} \tag{1}$$

The radius of curvature can then be related to the interfacial tension using the Young–Laplace equation, eq. 2.

$$\Delta P = \frac{2\gamma}{R_c} \tag{2}$$

The pressure can then be changed several times, thus giving several pairs of pressure and radius of curvature measurements. To obtain even more precise results than just calculating the interfacial tension from a single measurement, a graph of ΔP vs $\frac{2}{R_c}$ can be constructed, resulting in the value of the slope representing the interfacial tension in a much more precise fashion based on several measurements.

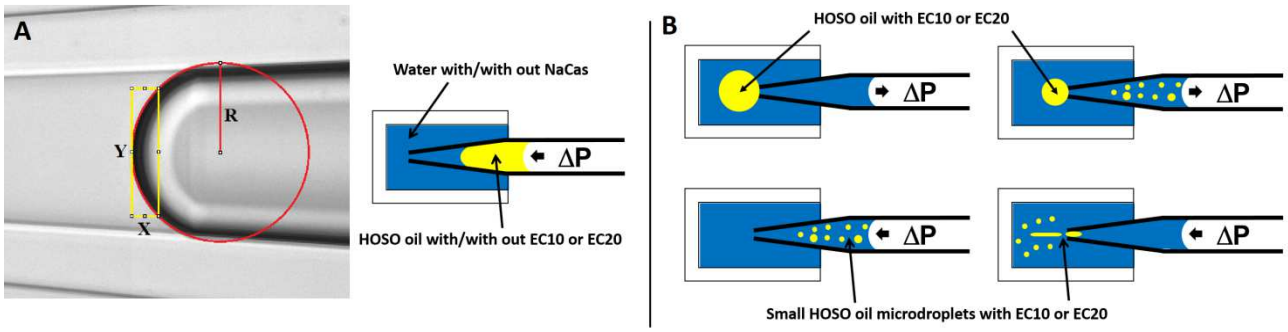


Figure 2. ***A)*** *Data acquisition from surface tension experiments. Example of the box (yellow) we use for extracting data for interfacial tension measurements. The box is placed so the left wall of the box just touches the meniscus, while the two right corners do the same. This takes place in the tapered part of the micropipette (interface between the blue and yellow phases), while making sure the oil phase extends into the parallel part of the micropipette.* ***B)*** *Micropipette manipulation of HOSO + EC emulsion in water. Larger microdroplets are located and caught with the micropipette. Increasing the suction pressure breaks the microdroplet into smaller microdroplets and sucks them into the micropipette. Reverting the suction pressure to blow the microdroplets back out deforms some of the microdroplets with diameters larger than the micropipette tip and microdroplet shape recovery or droplet-droplet interactions can be observed.*

2.5 Micropipette droplet manipulation

Coarse emulsions of EC oleogel-in-water were prepared as described above. Micropipettes with an o.d. of 5-20 µm were prepared as described by Duncan et al. (2004, 2006) and used for the experiments. As seen in **Figure 2B**, microdroplets of the oil phase with an appropriate size range (10 - 50 µm) were selected for experiments. These larger microdroplets were located and caught with the micropipette using a low suction pressure. Increasing the suction pressure broke the microdroplet into smaller microdroplets and sucked them into the micropipette. Reversion of the suction pressure to blow the microdroplets back out of the micropipette tip provided information about mechanical properties of individual emulsified microdroplets, such as deformation and shape recovery.

3. Results

3.1 Interfacial activity of ethylcellulose

The adsorption of surface active material at an interface between water and oil can be studied by measuring interfacial tension. That is, surface active components that accumulate in excess at the interface compared to bulk concentration will lower the interfacial tension. In order to deduce if EC accumulates at the oil/water interface, interfacial tension as function of EC concentration was measured as seen in **Figure 3**. The interfacial tension of pure HOSO and water was 26 mN/m and adding as little as 0.03 % EC (the lowest measured concentration) reduced the interfacial tension to approx. 10 mN/m. Adding more EC cP10 or EC cP20 had little effect as the data leveled off and, in any event, the measurements at high

concentration of EC were hindered by oil gelation. This behavior was also seen for other surface active polymeric systems like PEG (Gilányi, Varga, Gilányi, & Mészáros, 2006). The interfacial tension measurements show that EC is surface active and accumulates at the surface. For systems containing 0.3 % NaCas, a further reduction of the interfacial tension was measured with increasing concentration of EC, thus consolidating the surface active nature of EC even in presence of other surface active components (data not shown).

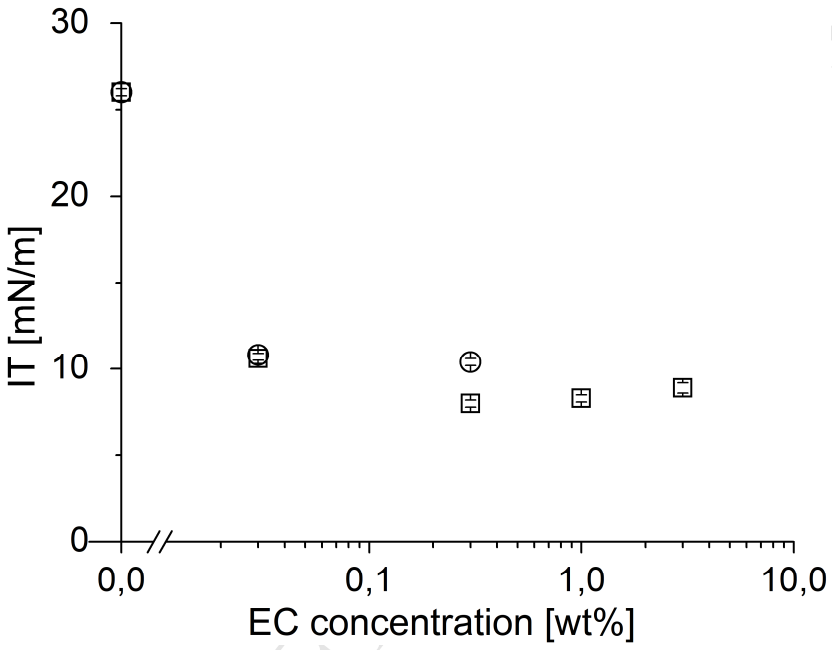


Figure 3. *Interfacial tension of EC-HOSO solutions against pure water as a function of EC concentrations: EC cP10 (square) and EC cP20 (circle).*

3.2 Oleogels and hot emulsified oleogels

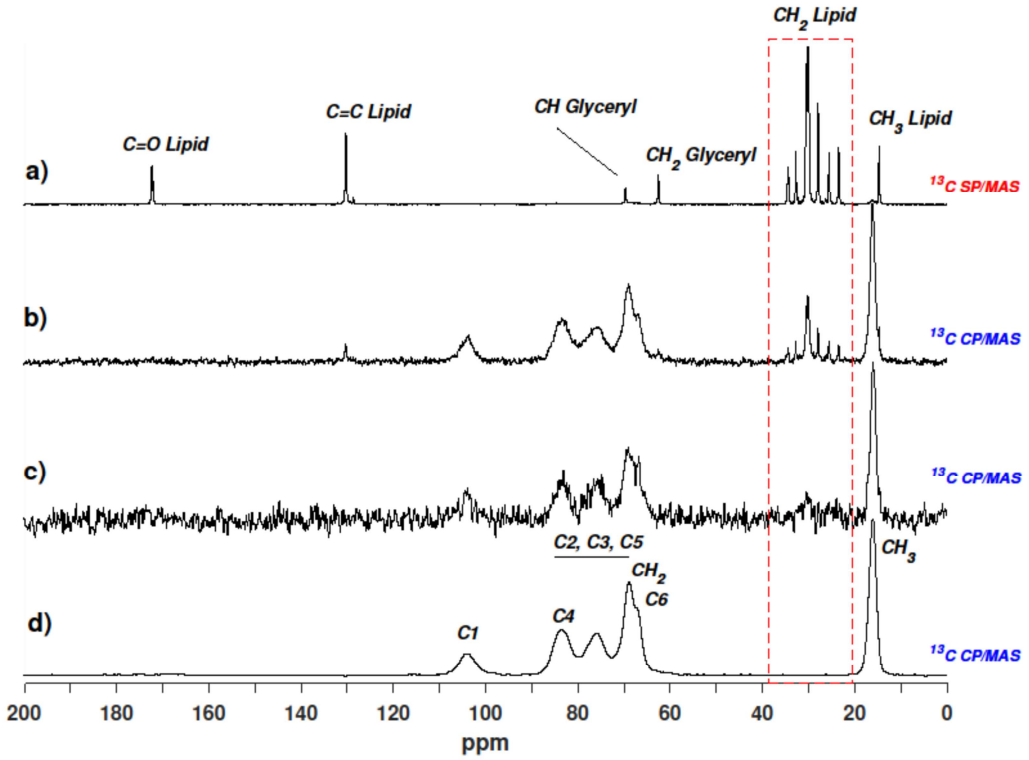
Next, we evaluated if and to what extent the oleogel could retain its gel properties when dispersed into an emulsion and put in contact with water. These experiments determined if and to what extent the physical characteristics of oleogels, especially their gelation properties, were changed as a consequence of emulsification and possibly uptake of water. These issues were addressed using ^{13}C-MAS NMR.

In this context, two NMR experiments were of particular importance. The carbon sites originating from the immobile regions of the sample were observed by ^{13}C CP/MAS NMR experiments, whereas all carbon sites were observed by ^{13}C SP/MAS NMR experiments. The reason for this selectivity is that polarization transfer from ^{1}H to ^{13}C by cross polarization (CP) requires non-vanishing hetero nuclear ^{1}H-^{13}C dipolar couplings and those are only present in the immobile regions. In the mobile regions such dipolar couplings will be

averaged out due to fast liquid or liquid-like motion of the molecules. Due to the selectivity of the CP/MAS data, only the SP/MAS data will provide a complete and quantitative description of the entire sample, whereas the CP/MAS data enables characterization of the immobile part only. **Figure 4a**) shows the ^{13}C SP/MAS spectrum of EC-oleogel containing 10 % EC cP20. As the oleogel contains nearly 90% HOSO and 10 % EC the ^{13}C SP/MAS spectrum shows that the oil constitutes the main part of the sample and the spectrum is dominated by the carbons resonances from the triglycerides of HOSO. **Figure 4d** shows a ^{13}C CP/MAS spectrum of the solid powder of EC and in this context it should be mentioned that CP/MAS and SP/MAS spectra were identical as all carbons in this samples are immobile. In this spectrum the carbon sites of glucose units as well as the ethoxy groups of EC were observed and assigned. Comparing **Figure 4a** and **4d** it is seen that only low intensity peaks from EC is visible in the SP spectra of an oleogel and the most obvious is the methyl resonance at 16.3 ppm.

The CP/MAS spectrum of the bulk oleogel (**Figure 4b**) is dominated by carbon sites from EC and thus this component has a low mobility in oleogels. Besides the broad resonances from EC, a range of narrow resonances with lower intensity originating from the lipids were present in the spectrum. Resonances from unsaturated, methylene and methyl carbons in the lipids were observed, whereas no carbonyl from the acid part of the fatty acids or carbons from the glycerol were detected. This indicates that the gelling mechanism primarily involves the acyl tails of the triglycerides rather than the glycerol and ester bond regions since the acyl tails are immobilized together with the EC. Comparison with the spectrum in figure 4a demonstrates that although the oleogel appears firm and solid-like when handling and deforming the material, only a minor fraction of the oil is immobilized in the EC oleogel. By integration the ratio of fatty acids to glucose unit were determined to be approximately 7 to 100.

The corresponding ^{13}C CP/MAS spectrum of EC-oleogels dispersed in water by heating the oleogels and applying high pressure homogenization at a temperature above T_{gel} shows NMR signals close to the noise level for lipid CH_2 carbons and thus indicating no immobilization of acyl tails of triglycerides and loss of the gelation effect of EC. No attempt was done to quantify the immobilized fatty acid chains as it was below the limit of detection.



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242 ***Figure 4.*** *^{13}C MAS NMR spectra of a) oleogel containing 10% EC cP20 applying single pulse SP/MAS, b) oleogel*
243 *containing 10% EC cP20 applying cross polarization (CP)/MAS, c) CP/MAS spectra of 10% oleogel-in-water emulsion*
244 *prepared using the hot emulsification method, d) CP/MAS spectra of pure EC cP20. The framed area shows the aliphatic*
245 *hydrocarbons of triglyceride acyl chains. Identical NMR spectra were obtained for the analogous samples made with EC*
246 *cP10.*

247 Gelation of bulk oil and oleogelator has been clearly detected macroscopically as solidification of the
248 material and it can be quantified in terms of gel hardness for example by texture analysis (Gravelle, Barbut,
249 Quinton, & Marangoni, 2014). Such macroscopic techniques and evaluations are not an option for micron
250 scaled dispersed droplets of oleogels in water. Instead evaluation of the properties of the dispersed oleogel
251 emulsion was performed by micropipette droplet manipulation. This unique technique enables studies of a
252 single microdroplet with a few microns of size while holding the microdroplet on the end of the
253 micropipette by a low suction pressure. Microdroplets for this purpose were prepared by shaking molten
254 EC oleogels and hot water. As shown by the times series of micrographs in **Figure 5,** a HOSO-EC
255 microdroplet in water was gently aspirated from the suspension and held at the mouth of the micropipette.
256 Upon the application of a low suction pressure, (at 1 s) the oil microdroplet started to move slowly into the
257 micropipette. After 5 s the interior of the microdroplet was drained and only an exterior crumbled up shell
258 remained. The microdroplet was restored by applying a small positive pressure thereby injecting the oil
259 back into the shell (10 s).

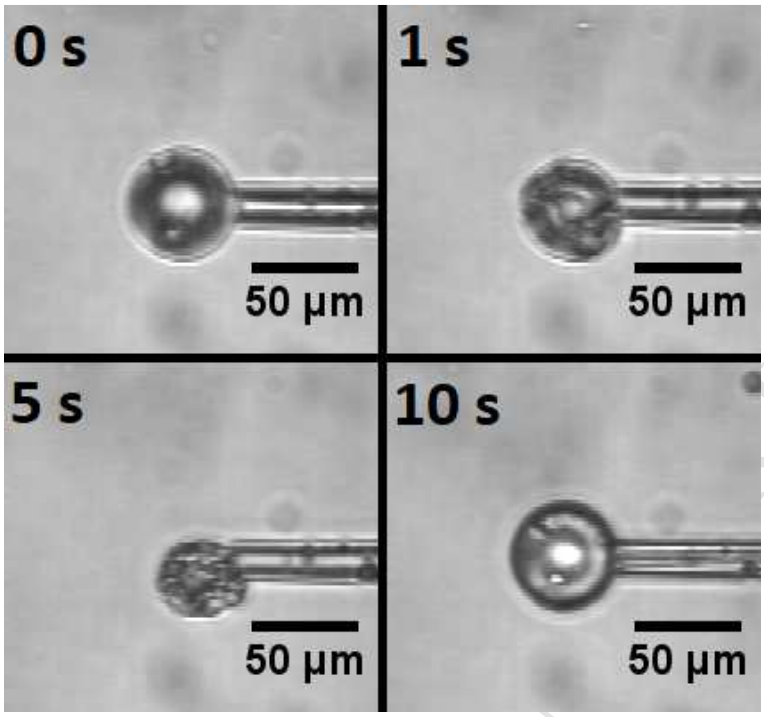


***Figure 5**. Video micrographs showing a HOSO-EC microdroplet in water, prepared by the hot emulsification method. At 1 s the oil starts to be aspirated by the micropipette and after 5 s the oil is drained from the microdroplet and only an exterior crumbled up shell remains. The microdroplet can be restored by reverting the suction pressure and thereby injecting the oil back into the shell (10 s).*

The fact that the internal oil can be separated from EC relatively easy and subsequently re-injected into the shell shows that the viscosity of the oil is rather low and consequently that some or all of the EC is most likely dispersed in the surrounding shell and thus not structuring the oil into a gel. This clearly showed that the interior of the oleogel was not a gel at all; it was simply a liquid oil microdroplet. Interestingly, the microdroplet had a fairly stable and relatively strong shell at its surface that remained intact during the draining of the interior and refilling. Hereby the micropipette study confirmed the observations by the ^{13}C MAS NMR data that oleogels are destroyed by hot emulsification.

3.3 Cold emulsified oleogels

It is a possibility that emulsification of the molten EC-oleogel in water enables the interfacially active EC to migrate to the interface of oil microdroplets and subsequently transform into solid-like surface material. From this viewpoint it was logical to attempt homogenization below T_{gel} at conditions where an EC-oleogel was formed and subsequently determine if access to water destroyed the gel.

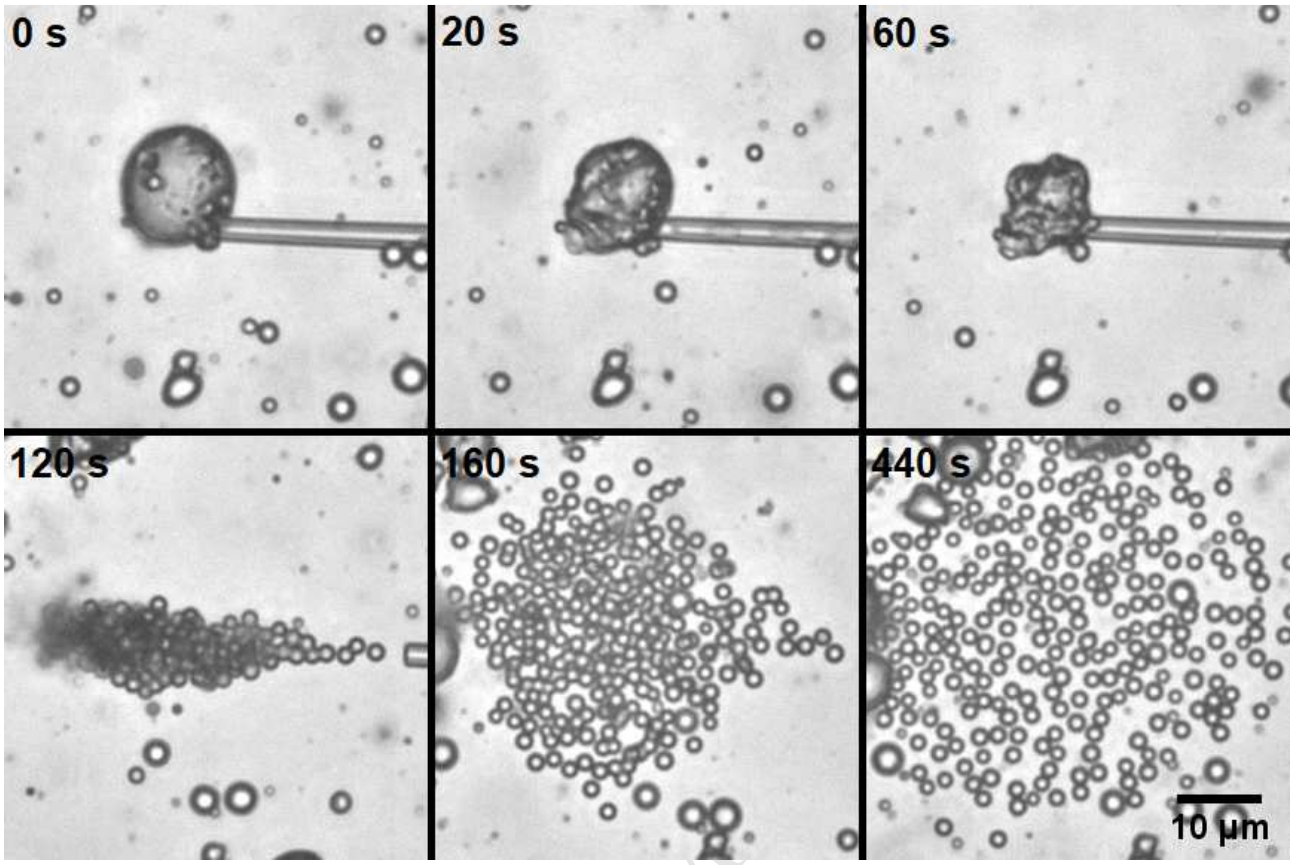


Figure 6. *Video micrographs showing microdroplets of HOSO-EC dispersed in water by the cold emulsification method. The flowability of the oil is low, thus it takes longer time to aspirate the oil with the pipette (60 s). During reinjection of the oil, the exterior non-imbibed material is detached from the micropipette and instead many small oil droplets that remain stable and intact for prolonged periods are dispersed.*

As shown in **Figure 6** one of the larger (15 µm diameter) cold-emulsified EC-oil droplets was gently aspirated and held at the tip of the micropipette. An increase in the suction pressure meant that material was again slowly aspirated into the micropipette, but this time the aspiration was considerably slower (60 s vs. 5 s for the hot emulsification method), indicating decreased flowability of the microdroplet interior. Unlike the exterior shell observed for the hot emulsified droplets, the non-imbibed material could not be refilled with oil, **Figure 6** (60 s). It merely detached from the pipette when attempting to reinject the oil. On the other hand, the material released from the pipette formed multiple smaller microdroplets that did not coalesce but remained stable despite close proximity and frequent collisions, **Figure 6** (160 s – 440 s). During material ejection into the chamber, tube-like morphologies were also observed which slowly within the time scale of one second slid back into the mother droplet, **Figure 7**. The slow time scale of the recovery of the shape indicates severe modification of the material compared to low viscosity oil. In comparison, in a previous study the recovery of droplets of a high viscosity liquid of 200 Pa s the time scale of recovery was in the order of 10 seconds (Tran-Son-Tay, Needham, Yeung, & Hochmuth, 1991). The

viscosity of pure HOSO is reported to be 0.067 Pa s (Quinchia, Delgado, Valencia, Franco, & Gallegos, 2009) and recovery of unstructured oil would be expected to happen within milliseconds and thus much faster than actually observed.

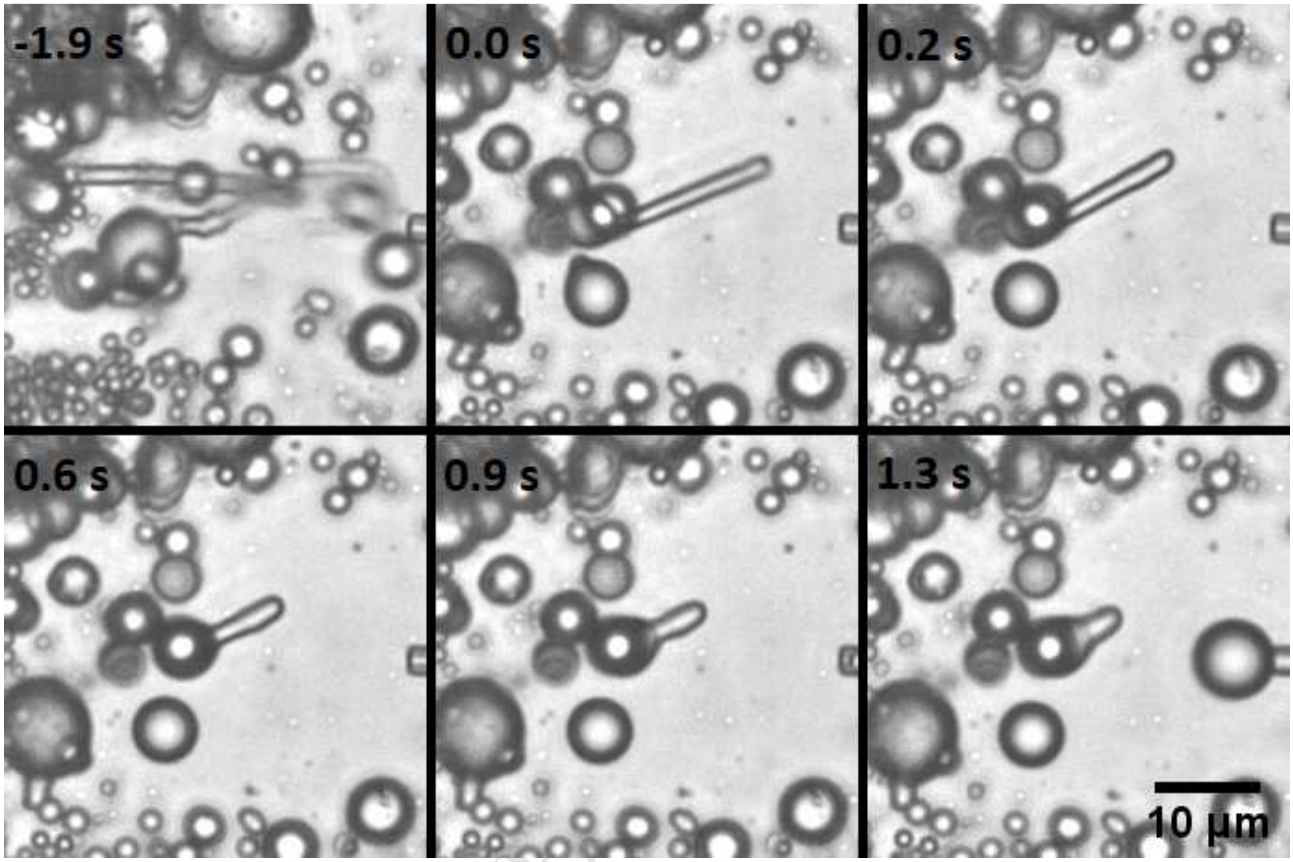
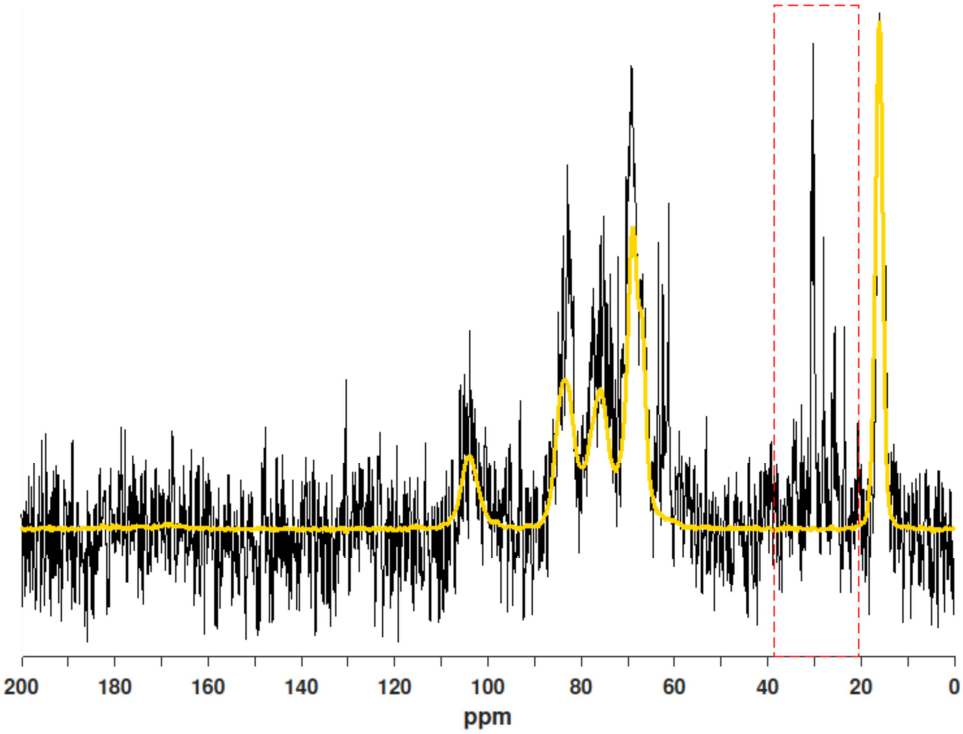


Figure 7. *Video micrographs showing formation of a HOSO-EC microdroplet by injection of oleogel that has been subjected to cold emulsification method. A fraction of the injected oleogel forms a tube-like morphology that gradually merges with the mother droplet within the time scale of seconds.*

The emulsions prepared by cold homogenization were studied using ^{13}C CP/MAS NMR as well. As for bulk EC-oleogels a small fraction of immobilized aliphatic carbon atoms of triglyceride acyl chains was observed, which indicates trapped triglycerides and thus formation of an oleogel as shown in **Figure 8**. By integration, the ratio of immobilized fatty acids to glucose units was determined to be about 8 per 100 glucose units and thus in the same order as bulk gels not in contact with water.

Identical ^{13}C CP/MAS NMR spectra for bulk EC-oleogels and oleogel emulsions combined with the slow recovery of oleogel microdroplets in micropipette experiments demonstrate that EC retains the ability to gel HOSO in emulsions if homogenization is performed at temperatures below the melting point of the oleogel.



311

312 ***Figure 8.*** *^{13}C CP/MAS NMR spectrum of 10% oleogel-in-water emulsion prepared using the cold emulsification method*
313 *(black). The NMR spectrum of EC is included as points of reference (yellow). The resonances of the immobilized*
314 *aliphatic methylene carbons from triglycerides are marked by the framed box.*

315 **4. Discussion**

316 EC solubilized in oil is surface active and accumulates at the oil-water interface as seen by measurements of
317 interfacial tension. The micropipette manipulation technique revealed precipitation of EC into a solid state
318 present at the microdroplet interface. The solid state of EC is used for tablet coating in the pharmaceutical
319 industry and in contact with water (e.g. by ingestion) EC forms water insoluble films suitable for retarding
320 drug release in the gastro intestinal tract (Siepmann, Wahle, Leclercq, Carlin, & Siepmann, 2008). The effect
321 of water on EC in oil is in a way non-trivial as conventional oil-soluble components (such as oil soluble
322 vitamins) are not precipitated by dispersing the oil into water as an emulsion. The observed behavior of EC
323 could be explained by the following mechanism. At low temperature, the stable form of EC is in solid form
324 and the true solubility of this solid EC is low. Oil gelation using EC brings this component into a non-
325 equilibrium state. The contact with water of molten gels accelerates the conversion into the stable solid
326 form and particles of this stable form accumulate at the oil/water interface through a Pickering mechanism
327 (Dickinson, 2010). This suggests that the shell is not a single coherent entity but rather composed of
328 multiple EC particles. However, further studies are needed in order to determine the structure of the shell.

In the present study, it was observed that formation of an oleogel minced into fine pieces and dispersed in water retained the oleogelating properties of EC. The mechanical properties of the interior of a microdroplet no longer reflected the flow characteristics of low viscosity oil, which was supported by the fact that a fraction of triglycerides was immobilized, as shown by ^{13}C MAS NMR studies. Actually, use of the cold emulsification method has been the common way to incorporate EC-oleogels in food products such as comminuted meat products and cream cheese (Barbut et al., 2016; Bemer et al., 2016; Zetzl et al., 2012), even though the gelation properties of EC in relation to processing methods has not previously been investigated.

The micropipette manipulation of cold emulsified EC oleogel microdroplets revealed slow recovery at a timescale of seconds. Reinjection of a microdroplet formed tube-like morphologies that slide back into the mother droplet rather than reverting to a compact more spherical shape. Such behavior can be related to surface solidification and shape recovery dominated by a solid surface layer (Kim, Costello, Duncan, & Needham, 2003). In this context, the micron scale structure of bulk EC oleogels must be discussed. Structure of EC oleogels on this length scale has to our knowledge only been reported in one paper (Zetzl et al., 2014). Here bulk oleogels are seen to contain oil pores of about 5 μm in diameter embedded in a rigid and more solid EC/Oil matrix material. When such inhomogeneous material is dispersed into droplets with diameters of 10-20 um, it is likely that the matrix rich in EC will wet the surface and apolar oil pores will be hidden in the interior of emulsion droplets. Such structure could explain the recovery behavior of tube morphologies and the imbibing incapability of the exterior part of the droplet using a micropipette, but this clearly needs to be confirmed by direct structural observations with an appropriate microscopic technique.

EC is added to the oil components in food products in order to obtain gelation of oil to mimic solid fats containing large proportions of saturated triglycerides. The present study reveals that care should be taken when assuming the same functionality of dispersed oleogels as in bulk oleogels and more special techniques need to be employed to assess the state of EC in dispersed oil phases. Micropipette manipulation can be used to observe droplets on micron scale in liquid emulsion systems and ^{13}C MAS NMR is shown to be useful to monitor immobilization of parts of the fatty acid chains even in complex and solid food matrices if other obscuring immobilized aliphatic carbon atoms are not present.

5. Conclusion

Mixing EC-oil mixtures with water at temperatures above the EC-oleogel set point will not result in an oleogel, but form a shell or a film at the interface of the oil droplets. The lack of gel formation was demonstrated by ^{13}C MAS NMR, and the presence of an interfacial shell by micropipette manipulation. In

contrast, if emulsions were prepared stepwise by initially making a set EC-oleogel and then disperse it into water at temperatures below the melting point of the EC-oleogels, then EC would still work as an oleogelator. This means that the oleogelating properties of EC can be utilized in O/W-emulsions when applying the proper preparation method, and this opens up for potential use of EC as an oleogelating agent in many emulsion-based food products.

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Highlights:

- Homogenization temperature is crucial for gelling properties of EC in O/W-emulsions
- EC forms a shell around oil droplets when emulsified at high temperatures
- EC-oleogel droplets remain gels when emulsified below melting temp of EC-oleogels
- ^{13}C MAS NMR is an excellent technique to study oleogels in complex food systems