

Article

Oil Droplet Coalescence in W/O/W Double Emulsions Examined in Models from Micrometer- to Millimeter-Sized Droplets

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Abstract: Water-in-oil-in-water ($W_1/O/W_2$) double emulsions must resist W_1-W_1 , $O-O$ and W_1-W_2 coalescence to be suitable for applications. This work isolates the stability of the oil droplets in a double emulsion, focusing on the impact of the concentration of the hydrophilic surfactant. The stability against coalescence was measured on droplets ranging in size from millimeters to micrometers, evaluating three different measurement methods. The time between the contact and coalescence of millimeter-sized droplets at a planar interface was compared to the number of coalescence events in a microfluidic emulsion and to the change in the droplet size distributions of micrometer-sized single and double emulsions. For the examined formulations, the same stability trends were found in all three droplet sizes. When the concentration of the hydrophilic surfactant is reduced drastically, lipophilic surfactants can help to increase the oil droplets' stability against coalescence. This article also provides recommendations as to which purpose each of the model experiments is suited and discusses advantages and limitations compared to previous research carried out directly on double emulsions.



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Keywords: DCTA; microfluidics; surfactant interaction; interfacial properties; PGPR; Tween 40

1. Introduction

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions are disperse systems, in which oil droplets within a continuous water phase are filled with smaller water droplets. This structure allows the encapsulation and targeted release of water-soluble substances e.g., vitamins or iron in foods [1] or proteins and peptides in pharmaceuticals and cosmetics [2]. To distinguish the continuous water phase from the water phase with the encapsulated substance, the inner water phase is commonly called W_1 and the outer water phase W_2 . Despite the large quantity of research that can be found on a variety of formulations for double emulsion applications, products with this structure are still seldomly found in the market because of stability problems.

Double emulsions tend to be more unstable than single emulsions due to the two differently curved interfaces [3]. These interfaces must be stabilized against three different coalescence paths, as shown in Figure 1 [4]. From the idealized starting point on the left side, the following coalescence mechanisms can occur: W_1-W_1 , the coalescence of inner water droplets; W_1-W_2 , the release of inner water droplets into the continuous phase; and $O-O$, the coalescence of (filled) oil droplets. To prevent or slow down coalescence, at least two surfactants are added. To keep the inner water droplets emulsified within the oil, most research is performed on the oil-soluble surfactants polyglycerol polyricinoleate (PGPR) and Span 80 [5]. For stabilizing the oil droplets, a variety of different water-soluble surfactants were applied and compared in different studies [6,7]. These ranged from short-chained ionic [8] and non-ionic [9] surfactants over synthetic polymers [10] to biopolymers, such as pectins and proteins [11]. Since the two different surfactants distribute at both interfaces and build mixed films, the resulting stability of a double emulsion is dependent on how well the two surfactants interact at the interfaces [12].

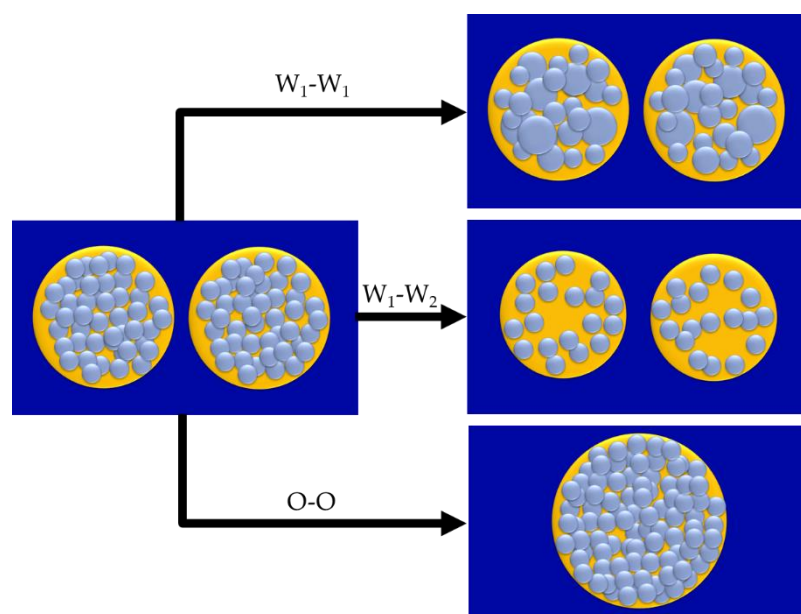


Figure 1. Possible coalescence paths in a monodispersed double emulsion. In the inner water phase (light blue), an active ingredient can be encapsulated. The oil phase (yellow) has a lipophilic surfactant dissolved within to stabilize the water droplets. The outer water phase (dark blue) has a hydrophilic surfactant to stabilize the oil droplets. This work focuses on the coalescence of oil droplets. Figure adapted from Leister and Karbstein [4].

Concerning the surfactant interactions, an often-discussed topic is whether the hydrophilic surfactant has a positive or a negative influence on the encapsulation efficiency. The general observation for PGPR-stabilized water droplets is that smaller hydrophilic surfactants are more likely to increase release than high-molecular-weight polymers [7,13,14]. Surprisingly, the outer interface, and thus O–O coalescence, is less commonly discussed in terms of the interactions between hydrophilic and lipophilic surfactants. Neumann et al. [15] published a study in which the influence of PGPR on the stability of oil droplets with different types of polyvinyl alcohol as hydrophilic surfactants is presented. Gülseren and Corredig [16] examined the properties of interfaces covered with PGPR and proteins in general, but focused more on the resulting stability of inner water droplets. Since oil coalescence is the main reason behind the macroscopic instabilities in double emulsions, such as changes in viscosity and creaming, it is important to understand the composition of the outer interface and the resulting stability against O–O coalescence as well.

The stability of the oil droplets is especially in focus when lower concentrations of hydrophilic surfactants are suggested to enhance the encapsulation efficiency [8,13,17]. Since some hydrophilic surfactants are known to decrease the inner water droplets' stability with higher concentrations [7,18], one way to produce stable double emulsion formulations could lie in the drastic decrease in the hydrophilic surfactants' concentration. According to previous studies, this could help to keep the inner water droplets stable, but the oil droplets might then be more prone to coalescence. Therefore, this study focuses on the question of how drastically the concentration of hydrophilic surfactants can be decreased without destabilizing the oil droplets completely.

One challenge that must be overcome to evaluate the stability of isolated oil droplets is to eliminate some of the parameters that affect droplet size. In emulsions in general, the droplet size directly after break-up is, apart from the stability against coalescence itself, dependent on the interfacial tension and viscosity ratio between the phases [19]. When comparing different formulations for double emulsions, the interfacial tension is dependent on the surfactant concentrations and their combination. The viscosity of the disperse W_1/O phase is a function of the filling degree of the oil droplets with inner water and can change when the encapsulated water phase is (partly) released during the process.

The change in the oil droplet size during storage is affected by two additional mechanisms apart from O–O coalescence: the release of the inner phase by W_1 – W_2 coalescence leads to shrinking droplets and the diffusion of water between the phases can lead to both, increasing and decreasing the droplet sizes, depending on the osmotic pressure in the W_1 phase [4]. For these reasons, the direct estimation of coalescence stability from measured oil droplet sizes in double emulsions is always associated with additional assumptions.

To overcome these limitations and to be able to perform experiments more quickly, several models of double emulsion have been developed in other studies. On the one hand, the general focus of these models of double emulsion is the decoupling of the production process from the instabilities. In all top-down emulsification processes (e.g., high-pressure homogenization, rotor–stator systems), the final droplet size is a result of break-up, collision and coalescence at the same time [20]. This results in a process-formulation combination-specific droplet size distribution. When the emulsion droplets are produced in a bottom-up approach (e.g., membrane emulsification or microfluidics), the droplet size can be more easily set to a fixed size [21]. On the other hand, the models can simplify double emulsions to single emulsions, where only one coalescence mechanism is possible, which allows the mechanism to be examined more easily [22]. Additionally, the time and material needed for the examination of each surfactant combination can be reduced.

When only examining the coalescence of oil droplets in double emulsions, the inner water droplets can be neglected [15]. When the lipophilic surfactant is added to the oil phase nonetheless, the molecular arrangement of the surfactant molecules at the O/W interface is likely to be the same, as should be the observed stability.

The most simplified version of a coalescence process is a single droplet coalescing at a planar interface with an experimental setup, often called “Diffusion and Coalescence Time Analyzer” (DCTA) [23]. This experiment was used previously to describe the coalescence of oil droplets [24] and water droplets [25] for different surfactant combinations. The time between the contact of the droplet with the interface is called coalescence time and can be used as a value to describe the stability of the film between two interfaces [4,26]. This experiment can be performed quickly and with little equipment, but the scatter of data points limits the conclusions as to significant differences in stability between two formulations.

With microfluidic chips of coaxial glass capillaries, monodispersed droplets with a variation in diameter of less than 5% can be produced [21]. The droplet fluid flows through a small tip, allowing droplet break-up in co-flow in the dripping regime [27,28]. To determine the stability of a monodisperse emulsion, the number of coalescence events can be calculated [29]. When two droplets of the same size and volume coalesce, their diameters increase by $\sqrt[3]{2} \approx 1.26$; when three droplets coalesce, their diameters increase by $\sqrt[3]{3} \approx 1.44$, and so forth. Therefore, the number of coalescence processes that lead to a certain droplet size distribution of a formerly monodispersed emulsion can be calculated. This allows the simultaneous measurement of the stability of many drops and the emulsion droplets can be stored in bulk for defined times, before taking a sample and measuring the number of coalescence events.

Emulsions produced with mechanical emulsification devices show rather broad droplet size distributions. When evaluating the stability of such emulsions, either the complete droplet size distributions are compared, or characteristic values are taken from the distribution. The Sauter mean diameter, or $x_{90,3}$, a characteristic value for the biggest droplets in the collective, which is responsible for creaming, among others, is often used as an example.

This work presents a comparison of the coalescence behavior of oil droplets from typical double-emulsion formulations in models of different size scales. The agreement of the models is demonstrated for oil droplet coalescence and the advantages and disadvantages of each system are discussed in terms of experimental effort and the validity of the results for real double emulsions. Using these experiments, the possibility of reducing the hydrophilic surfactant is discussed in terms of oil droplet stability. The results suggest that

lipophilic surfactants can help to stabilize oil droplets in W/O/W double emulsions when the concentration of the hydrophilic surfactant is too low for complete interface coverage. The findings on the stability of interfaces with multiple interfacial active compounds can also be transferred to other research fields. Enhanced oil recovery techniques, for example, also deal with the challenges of the break-up and coalescence of oil droplets within chemically complex systems [30–32].

2. Materials and Methods

2.1. Materials

The oil phase in the experiments was medium-chain triglycerides (MCT, Witarix 40/60), obtained from IOI Oleo GmbH, Hamburg, Germany. In the oil phase, the lipophilic surfactants PGPR from Evonik Dr. Straetmans GmbH, Hamburg, Germany and Span 80 from Carl Roth GmbH & Co. KG, Karlsruhe, Germany were dissolved at 1 wt%, respectively. Both lipophilic surfactants are widely used for the stabilization of W/O emulsion products. While Span 80 is a short-chained surfactant ($M_W = 429$ g/mol) with a head-tail structure, PGPR is a much larger polymeric surfactant ($M_W \approx 2500$ g/mol), with hydrophilic and lipophilic groups distributed throughout the molecule. For the water phase, demineralized water was used with the short-chained ($M_W = 1280$ g/mol) hydrophilic surfactant, Tween 40 (Carl Roth GmbH, Karlsruhe, Germany), added in different concentrations. Tween 40 was applied at 1 wt% as typical concentration of application and at 0.003 wt%, the averaged critical micelle concentration (cmc) of Tween 40 described in previous research [33–35]. Additionally, the stability of droplets without additional lipophilic surfactant and without Tween 40 was examined, resulting in nine different formulations for each experiment.

For the interfacial tension measurements, microfluidics and single-droplet experiments, the MCT oil was purified according to the method described by Dopierala et al. [36] before mixing with surfactants, since the impurities in the unpurified oil could have a great influence due to the small interfacial area compared to the sample volume [7].

2.2. Interfacial Tension Measurements

The interfacial tension of interfaces with different combinations of water and oil phases was determined using the pendant drop method (OCA 15 LJ, DataPhysics Instruments GmbH, Filderstadt, Germany) at a constant temperature of 23 °C. The interfacial tensions were obtained after an equilibration time of 60 min. All measurements were performed three times.

2.3. Single-Droplet Experiments (DCTA)

The measurement setup for obtaining the coalescence time of an oil droplet at an oil/water interface was first published by Taboada et al. [24] and is described in detail in their study. Oil droplets with 5 μ L volume were formed using a Hamilton syringe with a repeating dispenser (Hamilton Company, Reno, NV, USA). The droplet detached from the tip of the bent needle and floated to the oil/water interface. The measurement time is defined as the time between the first contact of the droplet at the interface and its coalescence. For each surfactant system, measurements were repeated for at least 30 droplets using at least three different cuvettes. Since the measured coalescence times of single droplets scatter statistically, the distributions are plotted in boxplot diagrams [7]. The median value t_{50} is used for comparing different surfactant systems.

2.4. Microfluidic Emulsions

Microfluidic emulsions were produced by a glass capillary system with coaxial glass capillaries. Figure 2 shows a draft of the setup. The construction used here was developed for the production of double emulsions, since three phases can be pumped into the chip simultaneously, and was used in previous studies [37,38]. In this study, the chip is used for single emulsion in co-flow break-up, with an additional downstream flow constriction, as described by Dewandre et al. [39]. The advantage of this setup for the production of

single emulsions compared to counter flow is that there is no need for complex surface modifications of the capillary tips to adjust the wettability [39].

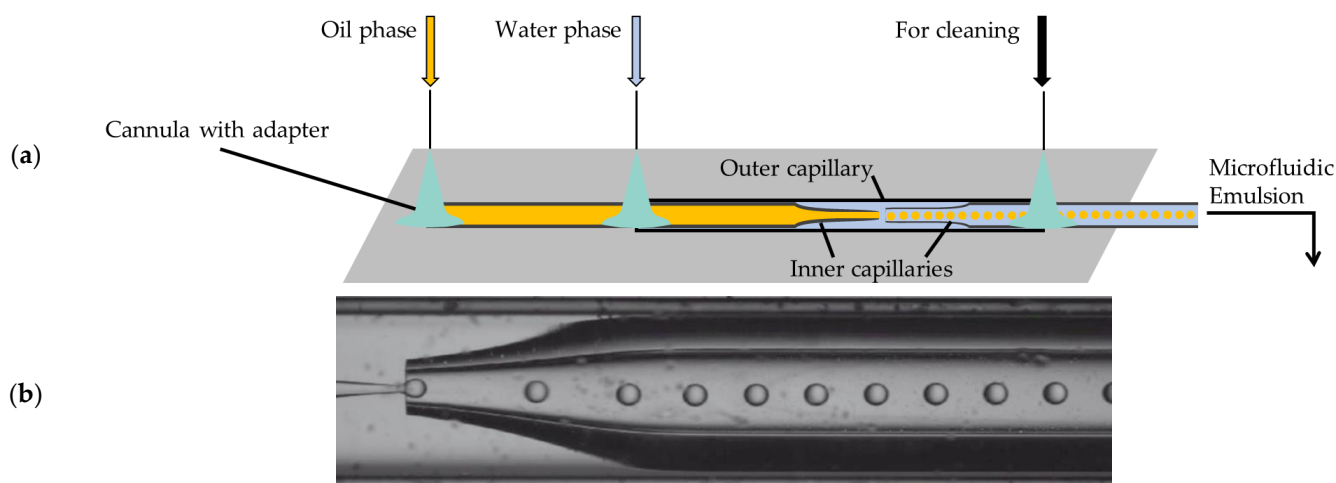


Figure 2. Draft (a) and micrograph (b) of the used microfluidic chip. The three cannulas are connected to syringe pumps, controlling the flow-rates. The left and right capillary have a diameter of 45 μm and 310 μm , respectively.

The oil phase flowed inside the conical capillary with the smaller tapered end ($d_1 = 45 \mu\text{m}$), while the continuous water phase flowed through the outer capillary in the same direction. The co-flow stream passed compellingly through the second capillary ($d_2 = 310 \mu\text{m}$) with the larger tapered end. There, oil droplets with highly monodisperse size distribution were formed. Regardless of the combination of different water and oil phases and their different interfacial tensions, oil droplets could be set to the same diameter ($d = 144 \mu\text{m}$; coefficient of variation (CV) < 4%) by adjusting the flow rates of both phases. The third cannula was used to empty the residual air in the setup prior to the production of microfluidic emulsions and to purge the system after the process. During the production of the monodispersed droplets, it was not in operation.

To build the setup, glass capillaries with tapered ends were fabricated. First, a round glass capillary (outer diameter: 1 mm, World Precision Instruments, Sarasota, FL, USA) was heated and cyclically pulled using a Micropipette puller (P-1000, Sutter Instrument, Novato, CA, USA). The tapered ends of the capillaries were polished to the desired diameter. In this study, two capillaries with diameter of the orifice of 45 μm and 310 μm were used. Next, the capillaries with tapered ends were assembled in an outer capillary (inner diameter: 1.05 mm, Boro Square, Atlantic International Technologies Inc., Rockaway, NJ, USA) and coaxially aligned using a microscope. The distance between the tapered ends of the inner capillaries was set to 75 μm . Syringe pumps (LEGATO[®] 100, KD Scientific, Holliston, MA, USA) were used to pump in the phases at specific flow rates. This process was captured live through a high-speed camera (DMK 33UX273, The Imaging Source Europe GmbH, Bremen, Germany).

2.5. Measurement of Droplet Diameter of the Microfluidic Emulsions

Microfluidic emulsions were stored in rolled rim bottles prefilled with continuous phase for either one day or for three months and then characterized for the stability of the droplets against coalescence. The microfluidic emulsions were pipetted on a microscope slide (Marienfeld Superior, Lauda, Germany). Due to the density difference, the oil droplets migrated to the center of the liquid surface and formed a hexagonally ordered group. Photographs were taken using a digital SLR camera (EOS 700D, Canon, Tokyo, Japan) equipped with a macro lens (Canon EF 100 mm 1:2.8 USM, Canon, Tokyo, Japan). For each combination of surfactants and each storage time, several microscope slides were

prepared so that at least 1200 droplets could be analyzed. On the microscope slide itself, no coalescence was observed.

Figure 3 shows three exemplary pictures of microfluidic emulsions after different numbers of coalescence events. Each droplet is labelled with its diameter in pixels, determined using a MATLAB code [40]. In Figure 3a, the emulsion still has a monomodal droplet size distribution. In Figure 3b, some of the droplets coalesced and the droplet diameter increased. In Figure 3c, the break-down of the emulsion is further progressed. To calculate how many coalescence events per 100 droplets occurred, the initial droplet number n_0 of the diameter d_0 that resulted in n_t droplets of the diameters d_i can be calculated by the Formula (1):

$$n_0 = \sum_{i=0}^{n_t} \frac{d_i^3}{d_0^3} \quad (1)$$

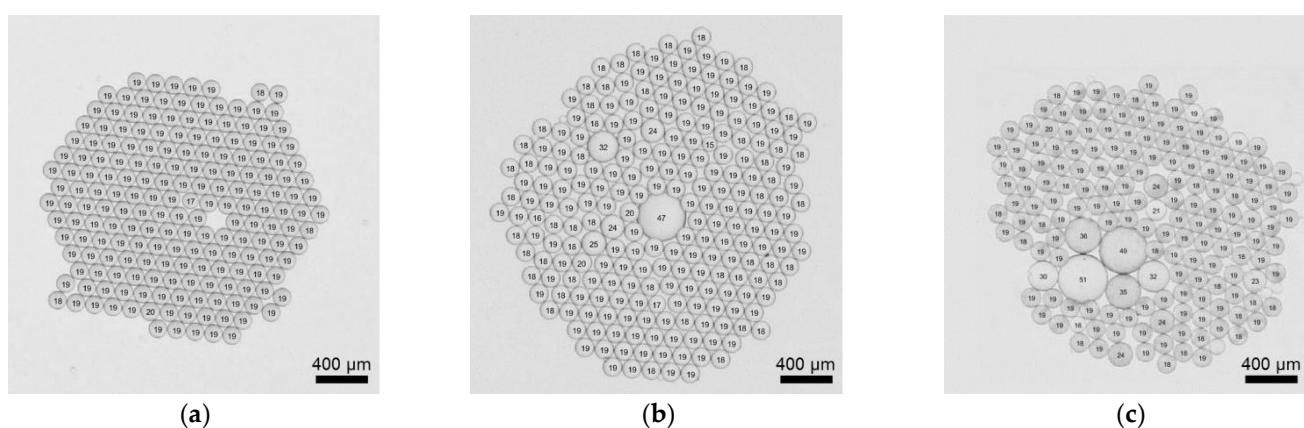


Figure 3. Exemplary micrographs of microfluidic double emulsions after different storage times. Each droplet is labelled with its diameter in pixels. (a) Emulsion droplets directly after production, the emulsion is monodispersed. (b) More stable formulation after three months. (c) More unstable emulsion after storage of one day.

The number of occurred coalescence events per 100 droplets c is then calculated from the remaining number of droplets n_t after a storage time:

$$c = \frac{n_0 - n_t}{n_0} \times 100 \quad (2)$$

In the Appendix information (Table A1), the measured diameters and calculated number of coalescence events from Figure 3 are given.

2.6. Top-Down Single and Double Emulsions

For the double emulsions, an inner emulsion with ($m_{W1}/m_O = 1/9$) was produced with a gear-rim disperser (IKA[®] magic LAB[®], IKA[®]-Werke GmbH & Co. KG, Staufen, Germany) at 15,000 rpm, corresponding to a maximum peripheral speed of 25.1 m/s for 5 min.

This W_1/O emulsion was dispersed in the outer water phase with ($m_{W1/O}/m_{W2} = 1/9$) using a colloid mill (IKA[®] magic LAB[®], IKA[®]-Werke GmbH & Co. KG, Staufen, Germany) for 5 min, with a gap width between the rotor and the stator of 0.318 mm. The rotational speed was set to 15,000 rpm, which corresponds to a maximum peripheral speed of 25.1 m/s. For the single emulsion, the same second emulsification step was performed with the surfactant-oil solutions as the disperse phase.

The droplet size distributions were measured using static laser diffraction (Horiba LA-950, Retsch Technology GmbH, Haan, Germany). Emulsions of each combination of water and oil phases were produced in duplicate and each sample was measured in

triplicate after different storage times. Images of the double emulsions were obtained with a microscope (Eclipse Ci-L, Nikon, Shinagawa, Tokyo, Japan).

3. Results

3.1. Interfacial Tension Measurements

The interfacial tension is a sensitive value with which to measure changes at interfaces. While it cannot be directly linked to the stability of the interface or the corresponding emulsion, it can show whether surfactants are adsorbed at an interface or not [7]. Table 1 gives the interfacial tension values after a 60 min equilibration time for all nine formulations examined in this work.

Table 1. Interfacial tensions of systems with different combinations of water and oil phase after 60 min equilibration time. The nine surfactant combinations shown here are used for all experiments.

Water Phase	Oil Phase	Interfacial Tension γ in mN/m
1 wt% Tween 40	Pure MCT oil	5.6 ± 0.2
	1 wt% PGPR	0.6 ± 0.1
	1 wt% Span 80	3.5 ± 0.1
0.003 wt% Tween 40	Pure MCT oil	10.6 ± 0.2
	1 wt% PGPR	1.7 ± 0.1
	1 wt% Span 80	4.2 ± 0.0
Pure water	Pure MCT oil	25.5 ± 0.4
	1 wt% PGPR	3.1 ± 0.0
	1 wt% Span 80	7.2 ± 0.1

Comparing all the values with 1 wt% Tween 40 in the water phase, both additional lipophilic surfactants decreased the interfacial tension further. This means that the additional adsorption of lipophilic surfactants to the interface occurred, which changed the properties of the interface. The same was observed for 0.003 wt% Tween 40 in the water phase.

The comparison between the PGPR of Span 80 at different Tween 40 concentrations shows differences between the formulations as well. Coming from measurements against pure water, the interfacial tension decreased with increasing Tween 40 concentrations, independently of the lipophilic surfactant in the oil phase. This shows that, here as well, the lipophilic surfactants did not adsorb at the interface alone, but the interfacial properties were a result of the combined adsorption. Therefore, all the examined formulations are different in interfacial composition and, accordingly, different droplet stabilities are to be expected.

3.2. Single Droplet Experiment

In Figure 4, the coalescence time distributions for the nine examined surfactant combinations are plotted in the form of a box plot diagram. The higher the coalescence time, the more stable the droplets against coalescence.

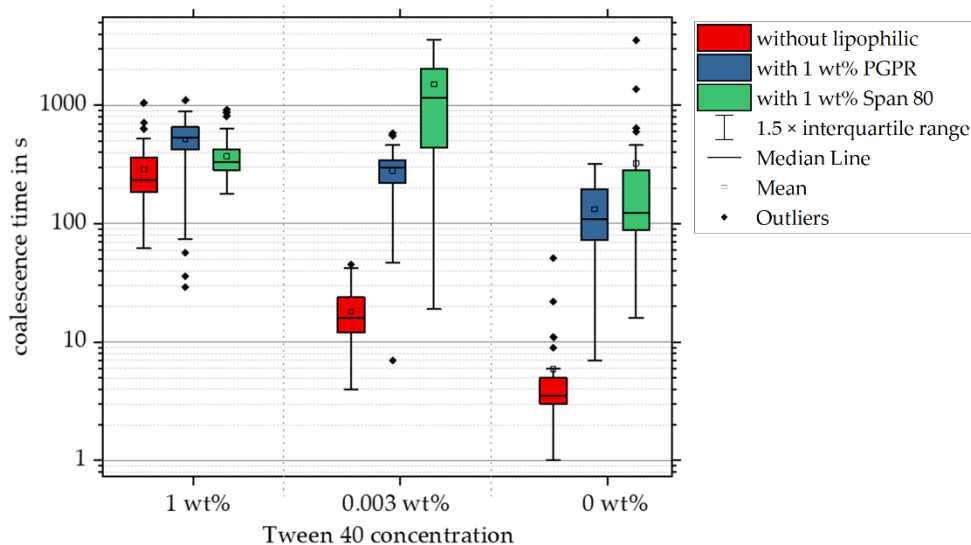


Figure 4. Coalescence time of single droplets in box plot diagram form. The box shows the middle quartiles, while the whiskers mark the upper and the down quartile. Additionally, the outliers and mean value are plotted. On the x-axis, the concentration of Tween 40 is varied. In the oil phase, either PGPR, Span 80 or no lipophilic surfactant were added. With decreasing concentrations of Tween 40, the stability of oil droplets decreases. Additional lipophilic surfactants can help to stabilize the water droplets.

Beginning with the left three boxes, droplets stabilized by 1 wt% Tween 40 are shown. For all the oil phases, with PGPR or Span 80 or without additional lipophilic surfactant, the coalescence times lay between 100 and 1000 s. Compared to other measurements with this measurement setup, the droplets were moderately stable. In other studies, some droplets were so well stabilized that no coalescence was observed at all within 30 min [24]. Here, the additional lipophilic surfactants increased the coalescence times slightly. The interface with two surfactants adsorbed increased the stability, or at least did not decrease it, as was found for the coalescence of water droplets [7,26].

When the Tween 40 concentration was reduced to 0.003 wt% (value given as the surfactants' cmc [33–35]), the stability of the droplets decreased, when no additional surfactant was added, by a factor of ten. The value of the interfacial tension measured after 60 min (10.6 mN/m instead of 5.6 mN/m) already indicates that the interface was occupied by fewer Tween 40 molecules at this concentration. This concentration effect can also be superimposed by slower adsorption times at concentrations around cmc [41]. Lower surfactant concentrations consequently lead to faster coalescence of single droplets [42]. The influence of additional lipophilic surfactant was more pronounced compared to the higher Tween 40 concentration. Both PGPR and Span 80 increased the stability of the droplets significantly. An explanation for this could be the lipophilic surfactant filling the free space at the interface left by the absent amount of Tween 40. The full occupation of the interface by a mixed surfactant layer showed a similar stability to the surfactant layer occupied by more Tween 40.

As expected, without any surfactant in the system, the droplets were completely unstable and the coalescence time dropped to below ten seconds. Again, the lipophilic surfactants increased the stability of the droplets. Still, there was a difference between the values without Tween 40 and with Tween 40 at cmc, showing that lipophilic surfactants alone cannot sufficiently stabilize oil droplets and that the stability of the combinations of Tween 40 at cmc with lipophilic surfactants resulted from mixed adsorption at the interface.

Even though the determination of coalescence times on single droplets is subject to large statistical deviations, this experiment shows interesting tendencies: in principle, the presence of an additional lipophilic emulsifier at the interface of oil droplets helps to stabilize them better against coalescence. This is particularly important when there is too

little hydrophilic emulsifier in the system to stabilize the oil droplets alone. Especially at very low concentrations of hydrophilic emulsifier (0.003% Tween 40), the longer resistance of the droplets to coalescence is assured just as well by the simultaneous presence of Span 80 or PGPR as by the sole application of 1% Tween 40. This offers interesting possibilities for double emulsions to reduce hydrophilic emulsifiers, which are known to destabilize inner water droplets.

3.3. Microfluidic Emulsions

The stability of the $d = 144 \mu\text{m}$ microfluidic droplets after one day and after three months is shown in Figure 5. Compared to other studies on the coalescence stability of microfluidic droplets, three months is a rather long storage time. Most other studies focus on coalescence on the microfluidic chip, where droplets are examined for some seconds or a few minutes [29,43–45]. The external storage of the droplets offers the advantage of examining more stable formulations that do not show any instabilities in the microfluidic channel. Additionally, the long observation time allows observations near or at equilibrium state at the interfaces, while short time stability strongly depends on the adsorption kinetics of the surfactants [20,46]. Approximately, the number of coalescence events between one day and ninety days doubled for all the formulations. This means that the coalescence rate decreased drastically over the storage period. This behavior was in agreement with the film drainage time for hard spheres and was observed in a similar form by Krebs et al. [47] for $100 \mu\text{m}$ drops.

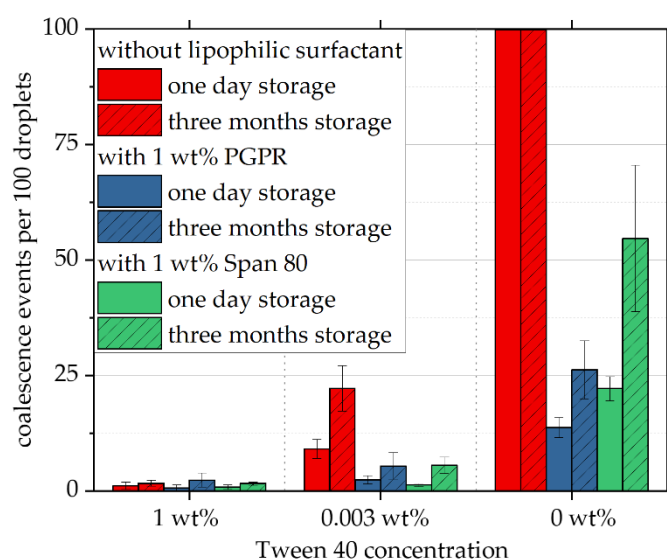


Figure 5. Number of coalescence events within the microfluidic emulsions ($d = 144 \mu\text{m}$; $\text{CV} < 3\%$) after one day and after three months. On the x-axis, the concentration of Tween 40 is varied. In the oil phase, either 1 wt% PGPR, 1 wt% Span 80 or no lipophilic surfactant were added.

For the microfluidic emulsion droplets with 1 wt% Tween 40, Figure 5 shows good stability values. Droplets of increased diameter were found in the samples but less than 1 out of 100 droplets coalesced within 1 day and less than 2 droplets coalesced after three months. Between the three formulations, no significant differences were seen.

At a reduced Tween 40 concentration of 0.003 wt%, the number of coalescence events increased. In the sample without lipophilic surfactant, approximately a quarter of the droplets coalesced within three months. With additional lipophilic surfactants, fewer coalescence events were observed the number was still below 7 coalesced droplets per 100 after 3 months. The two lipophilic surfactants showed a comparable stability within the measurement accuracy. The additional adsorption of PGPR and Span 80 obviously enhanced the droplets' stability.

Without Tween 40, the stability of the oil droplets was no longer given. Without lipophilic surfactant, complete phase separation was observed after 15 min and the maximum value of 100 coalescence events per 100 droplets was achieved. The additional lipophilic surfactants helped with the stabilization, but a large proportion of the droplets still coalesced. In these experiments, the sample with PGPR was significantly more stable than the sample with Span 80. While the lipophilic surfactants were also able to enhance stability without additional hydrophilic surfactant, the stability was strongly decreased in comparison with the samples with Tween 40.

Regarding the increasing error bars for measurement points with over 25 coalescence events per 100 droplets, the suitability of this method for very unstable formulations must be limited. Since the method assumes spherical droplets on the microscope slide, the deformation of huge droplets under gravity increased the error, at which point, many coalescence events occurred. Additionally, the number of measured droplets decreased drastically, especially since most droplets still had their initial diameter, while some droplets increased in size drastically (see Figure 3). With 1 droplet with over 50 coalescence events leading to it (see Table A1), the statistical errors from obtaining the sample with a certain volume increased.

3.4. Top-Down O/W Single Emulsions and W/O/W Double Emulsions

Single and double emulsions were produced with a colloid mill with identical process conditions and were measured for their droplet sizes directly after production and after two weeks of storage time. In Figure 6, the characteristic value $x_{90,3}$ of the droplet size distributions of the single emulsions (a) and of the double emulsions (b) are shown. Double emulsions without lipophilic surfactants could not be produced, since the inner emulsion separated instantly. The $x_{90,3}$ values for these formulations are therefore not plotted in Figure 6b.

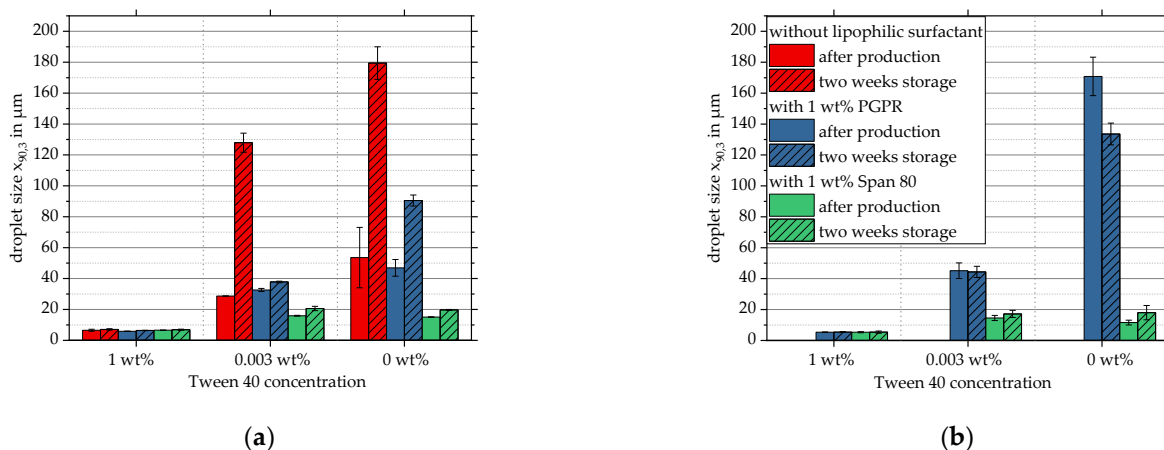


Figure 6. Changes in droplet size over storage time for O/W emulsions (a) and W/O/W emulsions (b) with the same W_2 and O phases. On the x-axis, the concentration of Tween 40 is varied. In the oil phase, either PGPR, Span 80 or no lipophilic surfactant were added. Double emulsions without lipophilic surfactant could not be produced, since they would instantly result in single emulsions; therefore, they are not shown.

With 1 wt% Tween 40, the oil droplets for both emulsions were below $10 \mu\text{m}$ and did not change their size within two weeks. Additional lipophilic surfactant neither changed the initial droplet size nor changed the stability during storage. Furthermore, no differences between single and double emulsions were seen.

With 0.003 wt% Tween 40, the droplet sizes were increased directly after the process for all the formulations. This might either have been influenced by changes in interfacial tensions (see Section 3.1) and, thus, decreased break-up, or by reduced coalescence stability

during the process. For the single emulsions, the droplet sizes after production were similar, whether PGPR or no lipophilic surfactant was added. Additional Span 80 led to smaller droplets. When the emulsions were stored for two weeks, differences between the addition and non-addition of lipophilic surfactant became more pronounced. Both lipophilic surfactants enhanced the coalescence stability during storage and only small changes in droplet size occurred during storage. The same trends were seen for the double emulsions: with Span 80, the initial droplet size was smaller than with PGPR and the droplet sizes did not change over time with either lipophilic surfactant.

Without Tween 40, the stability of the oil droplets strongly decreased without lipophilic surfactants; the same was observed when PGPR was added. The droplet sizes increased after production and were highly unstable after two weeks. Span 80 alone was also able to stabilize oil droplets 20 μm in diameter. For the double emulsions, the trend was more pronounced. With PGPR, the oil droplets were completely unstable; with Span 80, the droplets were still below 20 μm in diameter. The apparent reduction in droplet size for the samples with PGPR was due to the breakdown of droplets larger than 100 μm in the measurement device, which can lead to deviations.

To finally evaluate the suitability of the chosen surfactant combinations for the production of double emulsions, micrographs of the double emulsion are shown in Figure 7. The double emulsions were obtained directly after production and presented on the microscope slide without dilution. Longer storage times or dilution led to severe coalescence of the oil droplets for the emulsions without Tween 40 for both lipophilic surfactants.

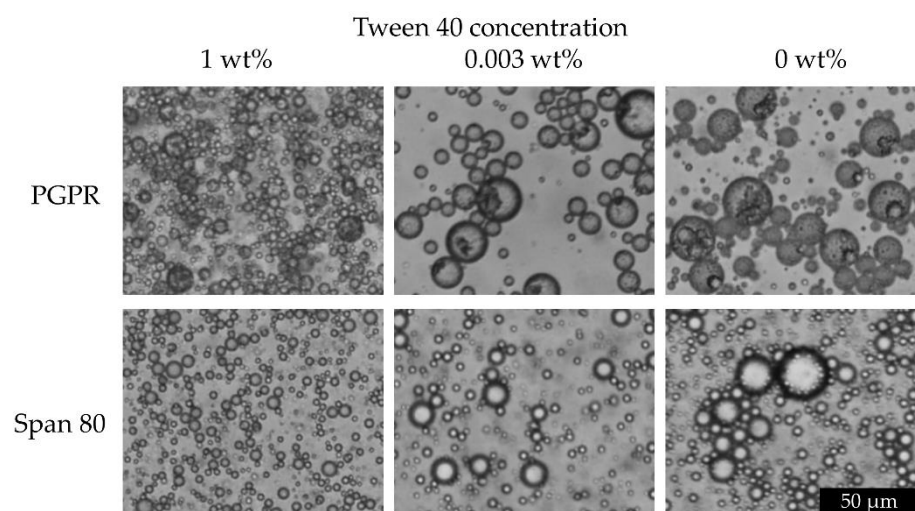


Figure 7. Micrographs of the double emulsions produced with 1 wt% PGPR or Span 80 and different Tween 40 concentrations directly after production. While the emulsions with PGPR as lipophilic surfactant showed encapsulated droplets and, therefore, a double-emulsion structure, the double emulsion with Span 80 had no inner droplets left. For both lipophilic surfactants, the oil droplet size decreased with increasing concentrations of Tween 40.

In the top row, double emulsions stabilized with PGPR are shown. With decreasing Tween 40 concentration, the oil droplet sizes decreased, as discussed above. The droplets were all grey, with some visible inner droplets, which means the double emulsion structure was intact. The Span 80-stabilized inner water droplets were no longer visible; the oil droplets were brighter, indicating that there were no inner water droplets. Pictures with higher magnification confirmed the absence of inner droplets. Although many studies using Span 80 for double emulsions are available [48–50], in our study, no double emulsion could be produced with this lipophilic surfactant.

4. Discussion

In this study, the effect of reducing the concentration of the hydrophilic surfactant on the stability of oil droplets in double-emulsion formulations was studied using different model experiments.

Interfacial tension measurements indicated that for all the surfactant combinations examined in this study, the composition of the interface differed, which resulted in different interfacial tensions. The influence of the interface composition on the stability of oil droplets was examined with model experiments of different size scales. The median coalescence time of single droplets at an interface varied between 3.5 s and 1200 s for the examined formulations. In principle, the presence of an additional lipophilic emulsifier at the interface of oil droplets increased the coalescence times for formulations with strongly decreased concentrations of hydrophilic surfactant. The wide scattering of the coalescence times prevented us from proving the statistical significance of the differences between the two lipophilic surfactants. The number of coalescence events in single emulsions produced by a microfluidic device showed values between less than 1 coalesced droplet per 100 droplets to 100 coalescence events per 100 droplets within minutes. Compared to the single-droplet experiment, the examination of microfluidic double emulsions increased the number of examined droplets from 30 to over 1000, allowing the performance of an improved statistical analysis. Finally, the droplet sizes of the single and double emulsions produced by mechanical emulsification were analyzed and the growth during storage was discussed. The value $x_{90,3}$ was chosen for comparison as a relevant parameter for the stability of an emulsion, since the biggest droplets were the most likely to lead to phase separation via creaming. The micrographs of the double emulsions emphasized the need for the complete examination of the double-emulsion system before recommending a suitable formulation. All the experiments suggested that the presence of a lipophilic surfactant helps to stabilize oil droplets even at very low concentrations of hydrophilic surfactant. Even though a similar behavior for PGPR and Span 80 was found concerning the stability of single oil droplets, the micrographs of the double-emulsion droplets revealed that the encapsulated water droplets were not stable when Span 80 was used as the lipophilic surfactant. This emphasizes the necessity of the different experiments proposed: while the model experiments can help to improve mechanistic understanding, only research on real double emulsion reveals interactions between all the possible instability mechanisms as they are equally important.

When comparing the information obtained from the different experiments, this study showed that at a typical application concentration of Tween 40 (1 wt%), the presence of additional lipophilic surfactants had no effect on the stability of the oil droplets. With a reduced Tween 40 concentration (0.003 wt% ~ cmc), the lipophilic surfactants increased the stability of the oil droplets against coalescence. Working without Tween 40 resulted in a strong decrease in oil droplet stability, confirming that lipophilic surfactants alone cannot stabilize O/W emulsions for longer than a day. In summary, Tween 40 provides satisfactory stability of oil droplets, even at low concentrations, when additional lipophilic surfactants such as PGPR or Span 80 are present. This opens up the possibility of reducing the concentration of Tween 40 in double emulsions drastically and still retaining relatively stable oil droplets. Since short-chained hydrophilic surfactants can destabilize encapsulated water droplets at high concentrations [7,18], the reduction of hydrophilic surfactant may offer the possibility of increased overall double-emulsion stability. Additionally, to reduce the concentration of surfactants as far as possible is a desirable goal from both ecological and economic viewpoints.

The changes in droplet stability were found to be similar in all three experiments at different size scales: single droplets (1000 μm), microfluidic O/W emulsions (150–500 μm) and (W/O)/W emulsions (1–50 μm). Table 2 shows the measured values obtained with the different experiments. For the sake of clarity, only the results of Tween 40 with PGPR after the short storage period are shown and discussed. Comparable trends were found after longer storage times and with Span 80 (see Tables A2–A4). The chosen storage times for

the microfluidic experiment (90 days) and the mechanically emulsified samples (14 days) differed. Because of the different droplet sizes, the time needed to develop significant differences between the samples differed.

Table 2. Comparison of the effects of the three different measurement methods on the different Tween 40 concentrations with addition of PGPR. For the microfluidic experiment, the values after one day for the emulsification experiments directly after production are given.

Tween 40 Concentration in wt%	Median Coalescence Time of Single Droplets t_{50} in s	Coalescence Events in Microfluidic Emulsion per 100 Droplets	Emulsion Droplet Size $x_{90,3}$ in μm	Double Emulsions Oil Droplet Size $x_{90,3}$ in μm
0	109	13.7	46.9	170.8
0.003	298	2.42	32.6	45.1
1	530	0.69	5.88	5.35

The general trend found in all the experiments is that a lower Tween 40 concentration results in increased coalescence. For the single-droplet experiment, an increase in the median coalescence time by a factor 5 was found when the Tween 40 concentration was increased from 0 wt% to 1 wt%. In the microfluidic experiment, the same Tween 40 concentration change led to a more than 10 fold decreased number of coalescence events. The increase in $x_{90,3}$ of single and double emulsions was changed by a factor of 8 and 30, respectively. The comparison shows that the same trends were seen in the different model experiments; however, the effects occurred to varying degrees.

5. Conclusions

All the methods studied generally work for the screening of surfactant combinations for double emulsions. They show how an additional surfactant or a change in its concentration affects the stability of oil droplets against coalescence. However, each experiment has its advantages and disadvantages and allows different effects to be illuminated more directly.

Interfacial tension measurements give information as to whether both surfactants adsorb simultaneously at the interface, without giving any information as to whether this affects droplet stability.

Single-droplet coalescence experiments allow the prediction of stability problems that may arise from specific surfactant combinations. No special laboratory equipment is needed to carry them out. However, this technique is very prone to distortion by small concentrations of impurities. Additionally, only rough approximations can be made, unless a huge number of repetitions are performed. The method is therefore rather time-consuming.

The observation of droplets produced by microfluidics unites the advantage of working with highly defined systems (droplet sizes) with the possibility of measuring hundreds of droplets simultaneously. Once the microfluidic process is established for the model system of interest, expanding the experiments to a wider variety of surfactant combinations works effectively. Still, handling microfluidic processes is challenging and the equipment is not widely available to research laboratories. While the method allows an optimum of mechanistic understanding to be gained, the method will be only used by research groups focusing on microfluidics. With the establishment of a suitable microfluidic process through which to produce double emulsions with multiple inner droplets, the complete break-down of a double-emulsion formulation can also be observed [51]. Accordingly, all three coalescence mechanisms can be tracked at once, eliminating the disadvantage that arises from using single emulsions for the description of double emulsions, namely that the optimizing of the stability against one coalescence mechanism might result in a worse stability against the other two.

In the case of single emulsions produced in laboratory-scale emulsification machines, the proximity to the application is good, as production and measuring instruments are

widely available. Furthermore, the transfer to the real system is easier. However, the (broad) distribution of droplet sizes in these systems makes an interpretation complex. Additionally, there is no possibility of tracking the other coalescence mechanism in double emulsions, such as W_1 – W_2 coalescence, while the other methods can be adapted for this coalescence effects as well.

Although the methods introduced in this research are highly effective at describing the one coalescence mechanism for which they are designed (O–O), the final step in formulation optimization remains the testing of real double emulsions. Each reduction in complexity might also reduce an important, yet unknown, effect on the stability.

While the models discussed in this paper can help to establish mechanistic understanding and support users with double-emulsion instability troubleshooting, the last step is confirmation in a real system. Besides oil droplet size, measured in this study, a microscopic picture should also be taken, and the encapsulation efficiency or release rate should be measured to assess the quality of the formulation.

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Appendix A

Table A1. Exemplary table for the analysis of coalescence events from the micrographs from Figure 3. Each droplet diameter was measured in pixels and the number of coalescence events necessary for this droplet size was calculated and normalized to the initial number of droplets necessary for this volume.

Diameter in Pixels	c_i	Figure 3a	Figure 3b	Figure 3c
		Number of Droplets Found		
19	0	185	171	128
20	0.2	1	2	1
21	0.4	0	0	1
22	0.6	0	0	0
23	0.8	0	0	1
24	1.0	0	2	3
25	1.3	0	1	0
26	1.6	0	0	0
27	1.9	0	0	0
28	2.2	0	0	0
28	2.6	0	0	0
30	2.9	0	0	1

Table A1. *Cont.*

Diameter in Pixels	Figure 3a		Figure 3b		Figure 3c
	c_i	Number of Droplets Found			
31	3.3	0	0	0	0
32	3.8	0	1	1	1
33	4.2	0	0	0	0
34	4.7	0	0	0	0
35	5.3	0	0	1	1
36	5.8	0	0	1	1
37	6.4	0	0	0	0
38	7.0	0	0	0	0
39	7.6	0	0	0	0
40	8.3	0	0	0	0
41	9.0	0	0	0	0
42	9.8	0	0	0	0
43	10.6	0	0	0	0
44	11.4	0	0	0	0
45	12.3	0	0	0	0
46	13.2	0	0	0	0
47	14.1	0	1	0	0
48	15.1	0	0	0	0
49	16.2	0	0	1	1
50	17.2	0	0	0	0
51	18.3	0	0	1	1
Number coalescence events per 100 droplets		0.1	10.8	28.8	

Table A2. Comparison of the effect of the three different measurement methods on the example of different Tween 40 concentrations the with addition of PGPR. For the microfluidic experiment, the values are from after 90 days and for the emulsification experiments, from after 14 days.

Tween 40 Concentration in wt%	Median Coalescence Time of Single Droplets t_{50} in s	Coalescence Events in Microfluidic Emulsion per 100 Droplets	Emulsion Droplet Size $x_{90,3}$ in μm	Double Emulsions Oil Droplet Size $x_{90,3}$ in μm
0	109	26.2	90.5	133.5
0.003	298	5.38	37.8	50.5
1	530	2.37	6.40	5.98

Table A3. Comparison of the effect of the three different measurement methods on the example of different Tween 40 concentrations with the addition of Span 80. For the microfluidic experiment, the values are from after one day. The values for the emulsification experiments directly after production are also given.

Tween 40 Concentration in wt%	Median Coalescence Time of Single Droplets t_{50} in s	Coalescence Events in Microfluidic Emulsion per 100 Droplets	Emulsion Droplet Size $x_{90,3}$ in μm	Double Emulsions Oil Droplet Size $x_{90,3}$ in μm
0	124	22.2	15.1	11.6
0.003	1165	1.32	15.9	14.6
1	330	0.810	6.59	5.36

Table A4. Comparison of the effect of the three different measurement methods on the example of different Tween 40 concentrations with the addition of Span 80. For the microfluidic experiment, the values are from after 90 days and for the emulsification experiments, they are from after 14 days.

Tween 40 Concentration in wt%	Median Coalescence Time of Single Droplets t_{50} in s	Coalescence Events in Microfluidic Emulsion per 100 Droplets	Emulsion Droplet Size $x_{90,3}$ in μm	Double Emulsions Oil Droplet Size $x_{90,3}$ in μm
0	124	54.7	19.7	18.7
0.003	1165	5.57	20.6	18.4
1	330	1.60	6.88	5.64

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