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Determination of the Dominating Coalescence Pathways in Double Emulsion Formulations by Use of Microfluidic Emulsions

Nico Leister * and Heike Petra Karbstein 

Institute of Process Engineering in Life Sciences–Food Process Engineering, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

* Correspondence: nico.leister@kit.edu

Abstract: In water-in-oil-in-water ($W_1/O/W_2$) double emulsions several irreversible instability phenomena lead to changes. Besides diffusive processes, coalescence of droplets is the main cause of structural changes. In double emulsions, inner droplets can coalesce with each other (W_1-W_1 coalescence), inner droplets can be released via coalescence (W_1-W_2 coalescence) and oil droplets can coalesce with each other (O–O coalescence). Which of the coalescence pathways contributes most to the failure of the double emulsion structure cannot be determined by common measurement techniques. With monodisperse double emulsions produced with microfluidic techniques, each coalescence path can be observed and quantified simultaneously. By comparing the occurrence of all possible coalescence events, different hydrophilic surfactants in combination with PGPR are evaluated and discussed with regard to their applicability in double emulsion formulations. When varying the hydrophilic surfactant, the stability against all three coalescence mechanisms changes. This shows that measuring only one of the coalescence mechanisms is not sufficient to describe the stability of a double emulsion. While some surfactants are able to stabilize against all three possible coalescence mechanisms, some display mainly one of the coalescence mechanisms or in some cases all three mechanisms are observed simultaneously.



Citation: Leister, N.; Karbstein, H.P. Determination of the Dominating Coalescence Pathways in Double Emulsion Formulations by Use of Microfluidic Emulsions. *Processes* **2023**, *11*, 234. <https://doi.org/10.3390/pr11010234>

Academic Editors: Luis Alberto Garcia Zapateiro, Ronaldo Gonçalves dos Santos, Ana M. Forgiarini and Sina Rezaei Gomari

Received: 17 December 2022
Revised: 30 December 2022
Accepted: 4 January 2023
Published: 11 January 2023



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Keywords: multiple emulsion; microfluidics; O–O coalescence; W_1-W_2 coalescence; W_1-W_1 coalescence

1. Introduction

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions are discussed for a variety of applications. Typical examples are fat-reduced food [1,2], encapsulation of sensitive substances [3–5] and targeted release of active ingredients [6–8] or as an intermediate step for the production of solid microcapsules [9]. Despite many efforts to find suitable formulations for these products, still few to no double emulsion-based products are available on the market. The lack of products is usually attributed to a lack of stability, although it is often not specified which of the possible instability mechanisms are most problematic [10]. When single emulsions are unstable, their droplet sizes change due to coalescence or diffusional processes. In double emulsions, oil and water droplet sizes can change. Additionally, the encapsulated phase can be released [11].

The coalescence of oil droplets (O–O) does not affect the encapsulation efficiency of the double emulsion, but it affects the properties of the emulsions, such as viscosity and color. When the oil droplets are not stable at all, the result can be phase separation between the outer water phase and the inner emulsion [12]. Another instability is the premature release of the inner water phase (W_1) and of the encapsulated substances within [13]. This release can either happen by the coalescence of inner droplets into the outer water phase (W_1-W_2) or by diffusion of the encapsulated substance through the oil phase [14]. When release happens via coalescence, the inner water and the encapsulated substance are released

simultaneously. When the release mechanism is diffusive, the amount of inner water can stay constant, while only the encapsulated substance is released or vice versa. Therefore, the encapsulation efficiency (how much of the encapsulated substance is inside the oil droplets) and the amount of inner water (disperse phase ratio $W_1:O$) are different values, which might develop differently over double emulsion storage [15]. Whether and how fast diffusive transport of the encapsulated substance to the outer water phase happens is linked to the solubility of the encapsulated substance in the oil phase and to the applied surfactants [16]. A distinction between release of the internal phase by coalescence or by diffusion is discussed by some authors [17–19]. However, many application studies only describe the encapsulation efficiency and do not examine the underlying mechanism, e.g., [20,21]. This is sufficient for the evaluation of a specific formulation but makes targeted improvements on double emulsion formulations difficult.

To prevent diffusion of water molecules between the inner and outer water phase, osmotic active substances are commonly added to the W_1 phase, balancing the capillary pressure of the curved interfaces with the osmotic pressure [22]. This balancing, however, leads to the requirement of constant droplet sizes for the inner water droplets over time. Due to the third coalescence pathway, coalescence of two inner water droplets with each other (W_1-W_1), the size of inner water droplets can increase. The resulting decrease in capillary pressure will lead to water transport from the outer water phase into the inner droplets. The osmotic swelling of the droplets may lead to osmotic swelling breakdown, the spontaneous and complete loss of the double emulsion structure [23]. Summarizing, only W_1-W_2 coalescence does directly release the inner water phase, but the other two coalescence mechanisms can also critically change the double emulsion properties and must also be suppressed.

Preventing the three coalescence mechanisms is mostly approached by adding at least two surfactants of different solubility [24]. A more hydrophilic surfactant stabilizes the outer interface (O/W_2) and a more lipophilic surfactant the inner interface (W_1/O). Whether a surfactant is well suited for the stabilization of an interface depends on many factors. The first mechanism to prevent coalescence is by preventing the approaching of two droplets by electrostatic forces or steric forces between the surfactants adsorbed at the interfaces. How strong these forces are and how far they can effectively work, depends, apart from the structure of the surfactant, on the ionic strength of the fluid and its pH. Additionally, the viscosity of the continuous fluid affects the coalescence stability. With higher viscosity, the film drainage happens slower and coalescence can be prevented. Finally, surfactants can stabilize thin films between two droplets. These semi-stable films form and can eventually rupture due to capillary waves. The role of the surfactants in preventing coalescence here is on the one hand by dampening the capillary waves by adding viscoelasticity to the interface [25]. On the other hand, the surfactants may not stabilize small holes in the film by supporting the hole curvature. This is described in the oriented-wedge theory and is an explanation why some surfactants work for W/O and some work for O/W emulsions [26]. The other reason for different surfactants for the two emulsion types is that the steric and electrostatic forces differ in the oil side and in the water side of an interface. Which of the stabilizing mechanisms is mainly responsible for emulsion stability depends on the examined system. A mechanistic understanding of coalescence is therefore complex, even for the case of single emulsions. For more detailed mechanistic insights to coalescence processes, other articles are available [27–29].

The two surfactants added in double emulsions do not only adsorb at the interface they are assigned for, but will distribute at both interfaces over time. The layer of the co-adsorbed surfactants leads to the final stability of a formulation against coalescence [30]. Up to date, it is not clear which surfactant combinations are particularly well or poorly suited for stable double emulsions and why there are such significant differences. The literature reports different findings on specific formulations, which will be shown as an example for SDS and W_1-W_2 coalescence. It is reported that double emulsions stabilized with PGPR and a polymer can be triggered to W_1-W_2 coalescence by the addition of SDS [17,31]. When SDS is

added to the water phase without additional polymers, no W_1 – W_2 coalescence is described in the literature [18], at least at low SDS concentrations [32]. For oils other than triglycerides and other lipophilic surfactants than PGPR, SDS is sometimes described as a suitable surfactant [33] and sometimes as a surfactant that triggers W_1 – W_2 coalescence [34]. The same, partly contradictory, results can be found for most commonly applied surfactants. This can be explained by the complex interactions of the multiple ingredients used in double emulsions. Changing one of the formulation parameters can influence the stability significantly. Product development is therefore often based on trial-and-error approaches for novel formulations [35] or step-by-step optimization of known formulations [36,37]. This is certainly partly due to the fact that a differentiation of the possible instability mechanisms is difficult to implement from a technical point of view.

The challenges with common measurement methods to describe the three coalescence pathways is shortly summarized here and can be found in detail in other works [11]. The inner W_1 droplets of a double emulsion are difficult to access by optical methods (microscopy, DLS, SLS) because the outer O/W_2 interface hinders direct access and the disperse phase content of the W_1 droplets is often too high for measurements [11]. For the outer droplet size, measurements can be conducted. However, for systematic studies on the suitability of different surfactant combinations, one should start with equal droplet sizes. This is very difficult to maintain since the surfactants influence the drop breakup behavior [38]. Additionally, the release of inner water during the second emulsification step can change the viscosity of the W_1/O phase, which will also affect the resulting oil droplet size [39]. For systematic research on W_1 – W_2 coalescence, the dependency of the choice of encapsulated substance on the encapsulation efficiency is also problematic [16,40]. Additionally, rather high release rates are reported during the second emulsification step, which again adds a formulation–process correlation [41]. Thus, it is not clear whether the formulation itself does not stabilize the droplets properly or if the choice of process conditions was not compatible with this formulation.

Microfluidic emulsions offer the opportunity to quantify the occurrence of the three coalescence pathways with one single approach. While emulsions produced with rotor-stator systems or high-pressure homogenizers come in a wide distribution of droplet sizes, microfluidic emulsions can be produced within 5% droplet size variation. Additionally, the same droplet sizes can be produced, while the viscosity and interfacial tension change, by adjusting the flow rates [42]. Starting with a monodisperse emulsion, the number of coalescence events can be calculated by examining the change of droplet sizes [42,43]. It has already been shown that microfluidic double emulsions allow for the observation of W_1 – W_1 coalescence [44] and W_1 – W_2 coalescence [45].

This research aims at introducing the method for tracking all three possible coalescence mechanisms simultaneously with a reasonable experimental effort. The coalescence stability of formulations with eight different hydrophilic surfactants in combination with PGPR as the lipophilic surfactant is described. Each formulation was produced with identical droplet sizes and the changes in structure were analyzed after 20 min, 80 min and one day. We show that this measurement approach can identify the dominant coalescence mechanisms which varies for different formulations. We suggest surfactant combinations that lead to more stable double emulsion droplets at the chosen set of parameters. As next steps, many additional parameters (e.g., pH, ionic strength, surfactant concentration, oil phase, droplet sizes) can be examined on their influence on the three coalescence pathways. Additionally, systematic testing of more surfactants can help gain a mechanistic understanding of how the chemical structure of surfactants determines the stability of double emulsion. This work acts as a first step in applying a measurement method for the simultaneous measurement of the three possible coalescence mechanisms.

2. Materials and Methods

2.1. Chemicals

The $W_1/O/W_2$ double emulsions were stabilized on the microfluidic chip with 1 wt% polyglycerol polyricinoleate (PGPR) in the oil phase. As oil, middle chain triglycerides (MCT) from IOI Oleo GmbH (Hamburg, Germany) at a viscosity of 25.4 mPa*s were used. To set the viscosity of both the inner (1.0 mPa*s) and outer aqueous phase (5.0 mPa*s), 5 wt% and 50 wt% of glycerol (purity 99.5%, SuboLab GmbH, Pfinztal-Söllingen, Germany) were dissolved in water, respectively. To stabilize the oil droplets off-chip, different hydrophilic surfactants were used. Sodium dodecylsulfate (SDS) and Tween 40 were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), Brij C10 and Brij 58 and CTAB were provided by Merck (KGaA, Darmstadt, Germany). Lutensol TO8, Lutensol TO15 and Pluronic PE 6800 ($M_w = 8000$ g/mol) were kindly given by BASF SE (Ludwigshafen, Germany). Lastly, two types of polyvinylalcohol, POVAL 28–99 ($M_w = 145,000$ g/mol) and POVAL 26–88 ($M_w = 160,000$ g/mol) (Kuraray Europe GmbH; Hattersheim am Main, Germany) were tested, but the results are not shown due to very poor stability. The dosage of the surfactants was kept constant at 1 wt%. This concentration is used as a starting point, in future experiments an adjustment of the dosage in respect to the cmc might be tried. Additional parameters (e.g., pH or ionic strength) were not examined in this work.

The surfactants were chosen to give examples of different functional groups and to examine the class of alcohol ethoxylates in more detail. The molecular weight and the chemical structure of the surfactants are given in Table 1. Additionally, a general classification of the surfactants is made and HLB values are given. The values for the ethoxylates were calculated after Davies [46]; since no value is available for the ammonium group of CTAB, in this case, the experimental value is given [47]. The calculations are given in Appendix A. The given HLB values vary in some cases from experimental values or HLB values calculated with other methods. Depending on the way of determining HLB values, the results differ. Although the surfactants show a wide range of HLB values, all of them are, in general, suitable to stabilize O/W emulsions and are commonly applied for this purpose.

Table 1. List of the examined hydrophilic surfactants. They are ordered in different classification groups. Additionally, the chemistry of the lipophilic and the hydrophilic groups are given. The number of ethoxylate groups is abbreviated with EO.

Hydrophilic Surfactant	Classification	Structure	Lipophilic Group	Hydrophilic Group	HLB
Brij 58	Nonionic	Linear	C16-alkane	20 × EO	6.0
Brij C10	Nonionic	Linear	C16-alkane	10 × EO	2.7
Lutensol TO15	Nonionic	Linear	C13-alkane	15 × EO	5.8
Lutensol TO8	Nonionic	Linear	C13-alkane	8 × EO	3.5
Tween 40	Nonionic	Branched head	C16-alkane	40 × EO	19.4
SDS	Anionic	Linear	C12-alkane	SO ₃ ⁻	40
CTAB	Cationic	Linear	C16-alkane	C ₃ H ₉ N ⁺	15.8
Pluronic	Polymer	Linear	polyoxypropylene	polyoxyethylene	–

2.2. Microfluidic Device

For the production of the double emulsion droplets, a glass capillary device with two break-up points was deployed. The setup and its function are described in detail by Leister et al. [45]. Figure 1a) shows a picture of the applied microfluidic device. In Figure 1b), micrographs of the break-up points and the transportation capillary are shown, and in Figure 1c), a schematic drawing of the device is given with colored phases for better visualization. In the first module, the inner W_1/O emulsion is formed and then transported to the second module. The capillary tips of the first and the second break-up are the two ends of one capillary, therefore the emulsion from the first break-up is transported without

any changes to the second break-up. In the second module, the W_2 phase is added and the double emulsion is formed.

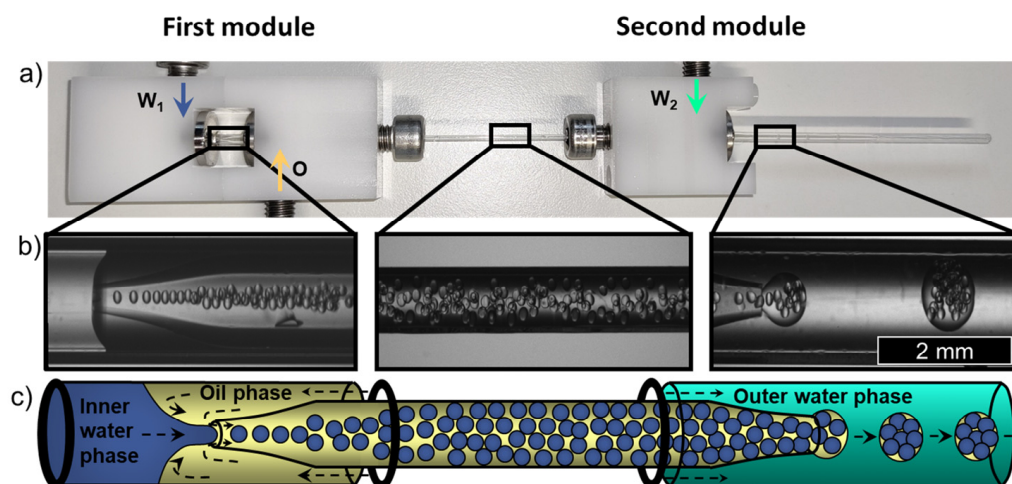


Figure 1. (a) Photography of the used combination of two microfluidic break up points. In the first module the inner emulsion is produced, in the second module is the double emulsion. (b) Microscopic images of the break-up points and the transport capillary between the devices. (c) A schematic drawing of the process. Reprinted from Leister et al. [45] with permission from Elsevier.

The capillary tips were made from 1.0/0.58 mm OD/ID capillaries (World Precision Instruments, Friedberg, Germany) with a micropipette puller (P-1000, Sutter Instruments, Novato, California). The orifice size was achieved by polishing the pulled capillaries to the desired sizes. The tips were treated with silanes supplied by Gelest Inc. (Morrisville, Pennsylvania): 2-[methoxy(polyethyleneoxy)6-9-propyl]tris(dimethylamino)silane was used for hydrophobic treatment at the first break-up and n-octadecyltrimethoxysilane was used for hydrophilic treatment for the second break-up. The positioning of the capillaries in the chip and the tubing of the fluids to the chip were made with so called LEGO-devices, as published by Bandulasena et al. [48].

The fluid flow rates were set with three digital syringe pumps (Legato 100, KD Scientific Inc, Holliston, Massachusetts) equipped with 25 mL and 50 mL high precision glass syringes (Cetoni GmbH, Korbussen, Germany). The droplet formation process was observed with monochrome cameras (DMK 33U) at each break-up point equipped with a $1\times$ lens (TMN 1.0/50) using the software IC capture (V2.5), all from The Imaging Source Europe GmbH (Bremen, Germany). The resulting droplet sizes of inner and outer droplets were determined using “Droplet Morphometry and Velocimetry” software [49].

2.3. Droplet Production and Analysis

All droplets examined in this work were produced with identical capillary sizes, flow rates and phases in the microfluidic chip and the break-up is shown in Figure 2. At the first break up point, W_1 droplets were produced by counterflow break-up at a $180\ \mu\text{m}$ capillary tip. At the second break up point, the outer droplets were formed in a co-flow break-up from a $320\ \mu\text{m}$ capillary tip into a $580\ \mu\text{m}$ collection capillary. The collection capillary was added from the original setup in Figure 1 to decrease the oil droplet size and thereby reduce the number of encapsulated W_1 droplets.

The flow rates were set to $Q_1 = 0.5\ \text{mL/h}$ and $Q_2 = 3\ \text{mL/h}$ at first break-up and $Q_1 + Q_2 = 3.5\ \text{mL/h}$ and $Q_3 = 50\ \text{mL/h}$ at the second break-up point. The inner phase was 5 wt% glycerol in water, the middle phase 1 wt% PGPR in MCT oil and the outer phase consisted of 50 wt% glycerol in water without any surfactant. The process produced oil droplets with a diameter of $376\ \mu\text{m}$ filled with $117\ \mu\text{m}$ water droplets, both with a variation of diameters smaller than 5%. The number of encapsulated droplets varied between 2 and 8 droplets. In an ideal setup, the number of encapsulated droplets would have stayed

constant. However, the inner emulsion droplets rearranged slightly during the transport from first to second break-up point. Therefore, at the second break-up, the water droplets did arrive more or less densely packed. The initial number of encapsulated droplets varied as a consequence, which has to be taken into consideration when interpreting the results.

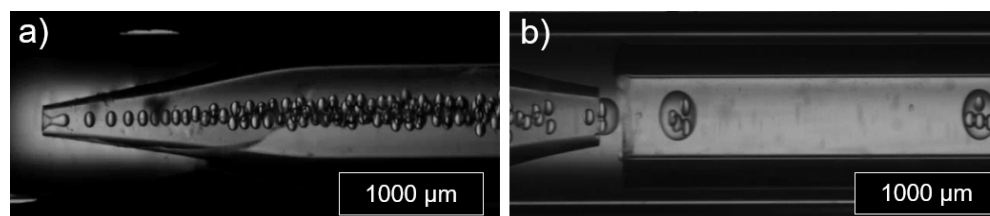


Figure 2. Production of W/O/W double emulsion droplets examined for coalescence in this work. (a) First break-up point. (b) Second break-up point.

The hydrophilic surfactants needed to stabilize the oil droplets against coalescence were not added on the chip, since they would alter the break-up by changing the interfacial tension [45]. Instead, the droplets were collected in different surfactant solutions. Since the oil droplets do not touch on the chip (see Figure 2b) it sufficed to add the hydrophilic surfactant afterwards. Each surfactant was dissolved at 1 wt% in water and the surfactant solutions were used to collect the double emulsion droplets. In the surfactant solution no glycerol was added. For all formulations, 2 mL of double emulsion from the microfluidic chip were collected in 18 mL of surfactant solution, resulting in 5 wt% glycerol in both W_1 and W_2 phase during storage. The osmotic pressure between W_1 and W_2 was therefore balanced, and no diffusion of water is to be expected. The surfactant concentration in the outer phase was correspondingly diluted to 0.9 wt% in the collection vial.

The postponed addition of the hydrophilic surfactant is potentially changing the surfactant arrangement at the interfaces. While in common processes both surfactants adsorb simultaneously, here the lipophilic surfactant is given some time (<30 s) to adsorb at the O/W_2 interface before the hydrophilic surfactant is added. Since the adsorption kinetics of PGPR are rather slow, even at high concentrations [50], it can be expected that the two added surfactants still form a mixed interface.

The double emulsion droplets were analyzed by taking photos with a single-lens reflex camera (EOS 700d, Canon, Tokyo, Japan) equipped with a macro-objective (Canon EF 100 MM 1:2.8 USM, Canon, Tokyo, Japan). The double emulsion droplets rise to the top of the water phase due to their lower density. When coming into contact with the water–air surface, the oil droplets tend to build an oil film and the phases separate to a layer of W_1/O emulsion and the W_2 phase. To prevent this additional instability mechanism, a measurement cell was developed. The droplets were put underneath a microscope slide with cavities (Marienfeld, Lauda-Königshofen, Germany), which was completely submerged in the W_2 phase. The droplets were then viewed under the microscope slide and pictures of the emulsion were taken from above. As soon as the droplets were in contact with the microscope slide, neither coalescence nor wetting of the oil to the glass slide were observed within one hour.

After defined storage times at room temperature, samples were placed under the microscope slide with a pipette. From the images, a random area was chosen with at least 50 oil droplets which were all characterized. For the inner and outer droplets, the sizes were measured and the number of inner droplets per oil droplets were counted. $O-O$ and W_1-W_1 coalescence were calculated from the change of droplet sizes and W_1-W_2 coalescence from the number of inner droplets in each oil droplet. To give an accurate number of the coalescence events, the values were corrected for the other two coalescence paths. For example, when two oil droplets coalesce, the number of encapsulated droplets doubles, and must therefore be divided by two, for the correct calculation of W_1-W_2 coalescence events.

2.4. Statistical Analysis

The measurements for the three coalescence mechanisms were repeated three times. This means that each datapoint shown in this study consists of the diameters of at least 150 oil droplets and, correspondingly, of at least 700 inner water droplets. While the number of analyzed droplets is small in comparison to data achieved with a method such as laser scattering, the monodispersity of the initial emulsion reduces the necessity of the analysis of thousands of droplets. From the increase in droplet size of this formerly monodisperse emulsion, the number coalescence events was calculated [42]. ANOVA was performed on the results at a level of 0.05 with Tukey test and Brown–Forsythe test for homogeneity of variance using OriginPro 2018.

3. Results

3.1. Qualitative Examination of Double Emulsions Stabilized with Different Hydrophilic Surfactants

In Figure 3, the photos of the double emulsion droplets used for the determination of coalescence stability are shown. The top picture shows the double emulsion droplets directly after break-up. Those droplets were collected under the microscope slide when coming out of the tubing of the microfluidic chip. The images show only a small area of the original pictures, which showed several hundred double emulsion droplets. Complete pictures of the droplets stabilized with Brij C10 are shown exemplarily in the supporting information (Figures S1–S3). The clippings shown here are chosen for the purpose of giving examples for the possible coalescence mechanisms. Statistically, the coalescence events occurred less frequently than depicted here.

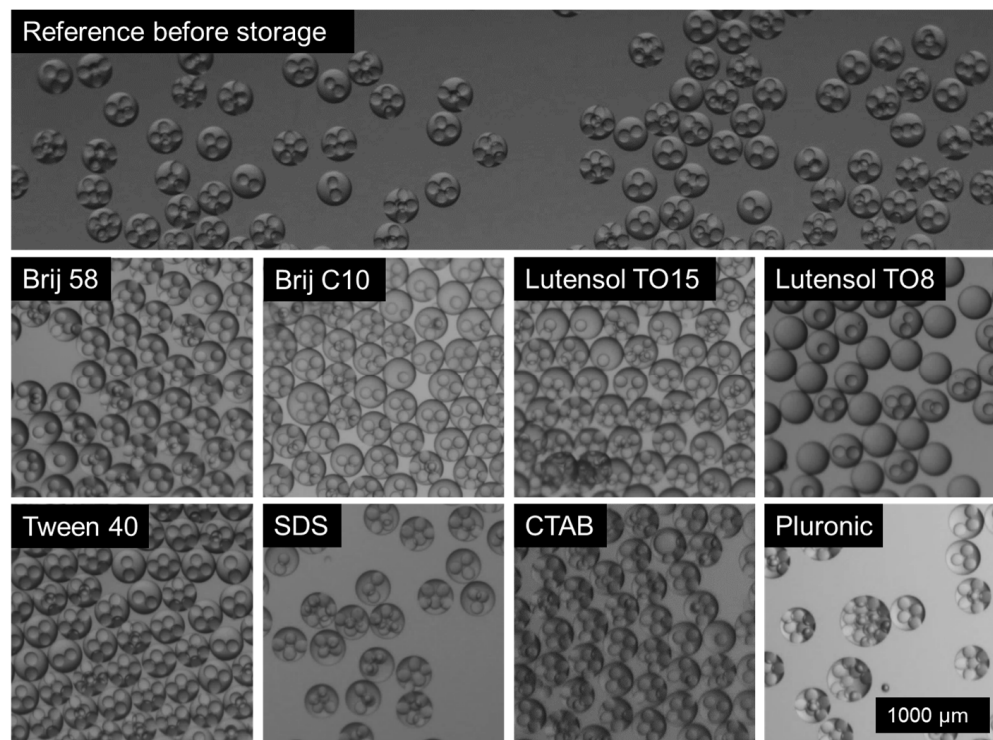


Figure 3. Photos of double emulsion droplets less than 30 s after microfluidic break-up (**top**) and after 20 min of storage in their respective surfactant solutions (**second** and **third** row). W_1 droplets were stabilized with PGPR for all samples. Droplets stabilized with Brij 58, Lutensol TO15, Tween 40 and SDS show near to no change. Emulsions with Brij C10 and Pluronic show O–O coalescence, with CTAB droplets show W_1 – W_1 coalescence and with Lutensol TO8 release of inner droplets via W_1 – W_2 coalescence occurs. The scale bar on the bottom right applies to all subfigures.

The reference picture directly after droplet production shows that the water droplets and the oil droplets are both nearly perfectly monodisperse. The number of inner droplets varies between two and eight. Due to way the microfluidic device is built, the water droplets are no longer homogeneously distributed in the oil phase when arriving at the second break-up point. Therefore, the number of encapsulated droplets varies. For the results, the average value of encapsulated droplets was used to determine the stability against W_1 – W_2 coalescence. The initial number of encapsulated droplets was identified by three methods. In the videos of break-up in the microfluidic channel, 4.80 water droplets per oil droplet were counted on average. Calculating the number of encapsulated droplets from volume flow ratio and droplet size ratio [45] gives a number of 4.74 inner droplets. In the reference picture without storage, 4.66 droplets were encapsulated on average.

In Figure 3, examples for the different coalescence mechanisms can be seen. A detailed comparison of the stability of the formulations will follow in Sections 3.3 and 3.4. The sample with Brij 58, Lutensol TO15, Tween 40 and SDS showed no coalescence within the first 20 min of storage time. It is noticeable that the oil droplets stabilized with SDS are way less densely packed than the other droplets. This can either be explained by different forces between the droplets, or, more likely, by the interaction of the droplet interfaces with the glass slide. For some surfactants, the droplets seem to be attached to the glass surface and do not move. For other surfactants, the droplets slid along the glass until they got into contact. The samples without any hydrophilic surfactant and the droplets stabilized with Pluronic also did not touch each other on the microscope slide. Whether the droplet got in contact or not, no coalescence event was observed on the microscope slide, even if the samples were left there for one hour.

On the pictures of the double emulsions stabilized with Brij C10 and Pluronic, coalescence of oil droplets was observed. While for Brij C10 only one droplet is larger and is the result of one coalescence event, the picture of the Pluronic stabilized emulsion shows two enlarged droplets, one built by one coalescence event, the other by the coalescence of three droplets. The sample stabilized with CTAB also looks rather stable, but on the top, one larger inner water droplet can be seen, which reveals W_1 – W_1 coalescence. Lastly, the double emulsion droplets stabilized with Lutensol TO8 are emptier than the other samples, showing the results of many W_1 – W_2 coalescence events.

The same trends increase for longer storage times. In Figure S4 in the supporting information, the same arrangement of pictures as in Figure 3 is shown for a storage time of one day. The breakdown of the double emulsion structure is progressed much more then, but showed the same trends as already observed at 20 min. Additionally, the oil phase got more turbid with longer storage time due to spontaneous emulsification of water in oil due to PGPR [51,52].

3.2. Coalescence of Oil Droplets (O–O Coalescence)

In Figure 4, the number of O–O coalescence events is plotted for the different hydrophilic surfactants after three timespans of storage. From left to right, the surfactants are grouped together for the nonionic surfactants (Brij, Lutensol and Tween 40) and the ionic surfactants (SDS and CTAB), followed by the polymer (Pluronic). For the formulations where not a single coalescence event was observed (e.g., Lutensol TO15), the value was set to 0.1 as minimum value. The bar for Pluronic after one day was set to 100, since the oil phase did separate completely in the collection vial, meaning that all oil droplets coalesced.

For six of the short chain hydrophilic surfactants, no significant O–O coalescence events were found for one day of storage (ANOVA see Table 2 in Section 4). Only for Brij C10 was oil droplet coalescence significantly higher. The other six short chain surfactants were nearly completely stable against this coalescence mechanism. For Lutensol TO15 and Tween 40, not a single coalescence event was detected within the measurement period. The other surfactants showed some coalescence events. Since only 50 oil droplets were measured for each determination, numbers below 2 are the result of only one coalescence event observed. A high stability for oil droplets with PGPR and Tween 40 was already

shown, even for Tween 40 concentrations below cmc [42]. Apart from this study, little data is published on the coalescence of oil droplets in W/O/W double emulsions.

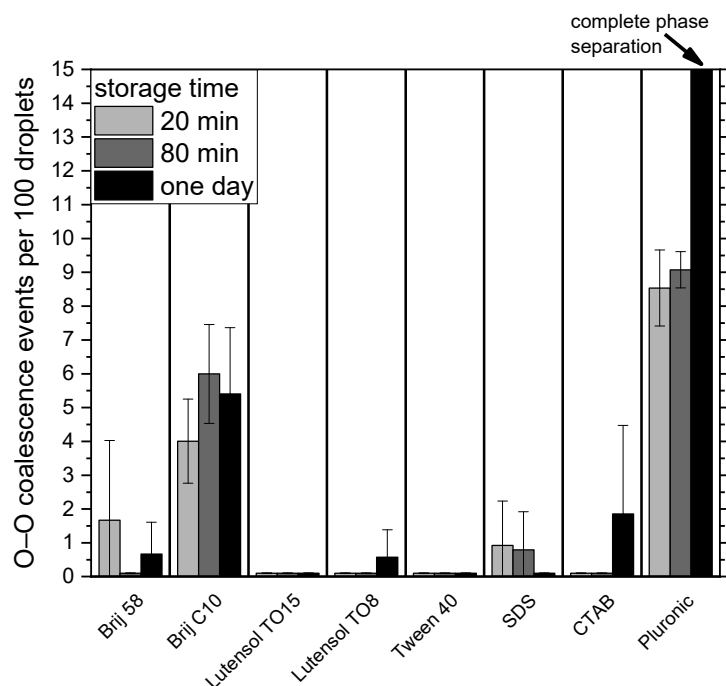


Figure 4. Number of O–O coalescence events normalized to 100 droplets with 0.9 wt% of different hydrophilic surfactants in W_2 and 1 wt% of PGPR in O. Double emulsions with Brij C10 and Pluronic show most oil droplet coalescence, the other samples are comparatively stable. With Tween 40 and Lutensol TO15, the emulsions show not a single coalesced oil droplet for all storage times investigated.

For Brij C10 and Pluronic, significant coalescence of oil droplets was observed. With 4 to 7 coalescence events per 100 droplets with Brij C10 and between 7 and 10 with Pluronic, clear trends are seen. While the Brij C10 emulsion was, after one day, still stable enough to measure droplet sizes, Pluronic stabilized $W_1/O/W_2$ double emulsion droplets separated completely in a W_1/O emulsion and W_2 phase. The reason for Brij C10 being more unstable than the other short chain surfactants can be found in its low HLB value of 2.7. It is the most lipophilic alcohol ethoxylate examined and is therefore less suitable for the stabilization of O/W emulsions.

The instability of the oil droplets with Pluronic as a polymer was also seen with two additional polymers. Two types of the amphiphilic polyvinyl alcohol were additionally tested. Both polyvinyl alcohols could not stabilize the oil droplets well enough for any samples to be taken after 80 min and the results were therefore not included in this work. All three polymers, however, are reported to be suitable for the production of double emulsions [11,53–55]. However, the stabilizing mechanism of the polymeric surfactants might rely on a viscosity increase of the W_2 phase and the formation of a stabilizing network in the continuous phase rather than on stabilizing O droplets in direct contact [12]. Additionally, decreasing the concentration of around 5 wt%, as commonly found in application, to 1 wt% in this study might have resulted in a weakened network and thus a decrease in oil droplet stability. In future works, different surfactant concentrations should be tested additionally. Nonetheless, it can be concluded that the interface stabilizing properties of the examined polymers were inferior to those of the short chain surfactants.

3.3. Release of Water Droplets (W_1 – W_2 Coalescence)

Figure 5 shows the number of W_1 – W_2 coalescence events per 100 inner droplets for the examined hydrophilic surfactants over time. The double emulsion droplets stabilized with

Brij 58, Lutensol TO15, Tween 40 and SDS showed very good resistance against W_1 – W_2 coalescence with only little release of the inner phase.

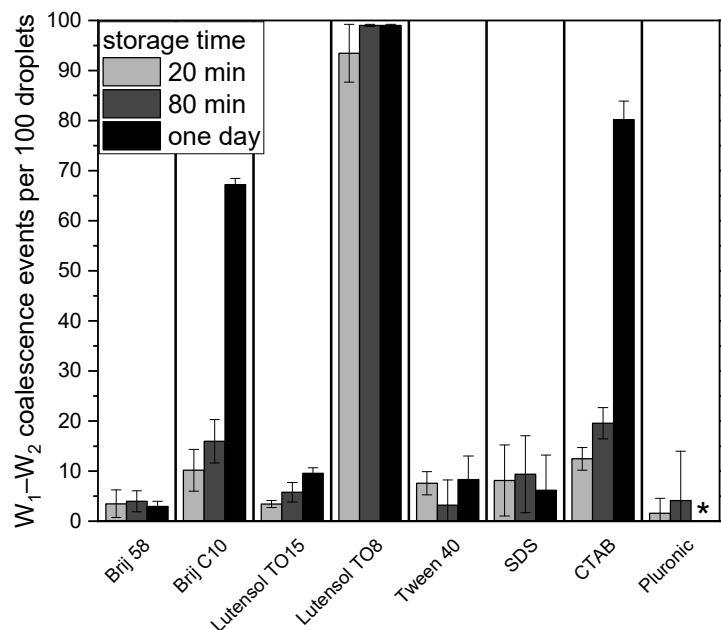


Figure 5. Number of W_1 – W_2 coalescence events, calculated from the number of encapsulated water droplets in each oil droplet. Droplets were stabilized with 0.9 wt% of hydrophilic surfactant in W_2 and 1 wt% of PGPR in O. Most surfactant combinations show good resistance against W_1 – W_2 coalescence, except Brij C10, Lutensol TO8 and CTAB. The last bar (marked with an asterisk) for Pluronic could not be measured, since no oil droplets were left due to O–O coalescence.

With Brij C10 and CTAB as hydrophilic surfactant in the double emulsion, stability against W_1 – W_2 coalescence is reduced. After 20 min and 80 min, the number of inner droplets is already slightly decreased. After one day, formulations with those two surfactants lost more than half of the encapsulated droplets. The formulation with Lutensol TO8 is very unstable against W_1 – W_2 coalescence and shows nearly complete W_1 release after 20 min. After 80 min, not a single encapsulated droplet was observed.

The inner droplets of the double emulsion stabilized with Pluronic were stable within the first 80 min, however, the instability of the oil droplets did not allow for measurements after one day, since no more oil droplets were left. It was observed that plenty of water droplets still were present in the oil film, hinting at good W_1 – W_2 stability, but it was impossible to calculate how many water droplets per oil droplet survived.

No clear link between the chemical structure of the hydrophilic surfactant and the stability against W_1 – W_2 coalescence was found. Lutensol TO8 and Brij C10 both have a relatively short hydrophilic chain and therefore a low HLB value. They showed more W_1 – W_2 coalescence than the corresponding surfactant Lutensol TO15 and Brij 58. In single droplet experiments, no such trend was found for the alcohol ethoxylates, they showed similar W_1 – W_2 coalescence stability with different chain lengths [56]. CTAB has a significantly higher HLB value than the alcohol ethoxylates and shows, nonetheless, W_1 – W_2 coalescence. Therefore, the tendency to W_1 – W_2 coalescence cannot be reduced to the HLB value alone.

3.4. Coalescence of Water Droplets (W_1 – W_1 Coalescence)

In Figure 6, W_1 – W_1 coalescence is depicted. All W_1 droplets were stabilized with PGPR from the beginning. The hydrophilic surfactant was later added to the W_2 phase. In general, it is considered that the hydrophilic surfactants cannot directly adsorb at the W_1 /O interface and change the stability of W_1 droplets. However, hydrophilic surfactants are

reported to diffuse through the oil phase to the W_1/O interface over time and destabilize it [26]. Again, the value for no coalescence event is set to 0.1 for illustration. Lutensol TO15 samples showed nearly no W_1-W_1 coalescence at all. Additionally, SDS did not interfere significantly in W_1-W_1 droplet stability within the first day of storage. For the samples stabilized with Brij 58, Tween 40 and Pluronic, some of the inner droplets coalesced. Brij C10 and CTAB samples were the most unstable for this coalescence mechanism. For Lutensol TO8, no inner droplets of increased size were observed, for the reason that all inner droplets were released rapidly. It is interesting that the same surfactants that triggered W_1-W_2 coalescence also showed W_1-W_1 coalescence (Brij C10 and CTAB). It can be expected that for double emulsions stabilized with Lutensol TO8, W_1-W_1 coalescence also occurs and is just not observed here due to the fast release. Therefore, the hypothesis can be postulated that the stability against coalescence of two water phases in double emulsions is similar whether it concerns W_1-W_1 coalescence or W_1-W_2 coalescence.

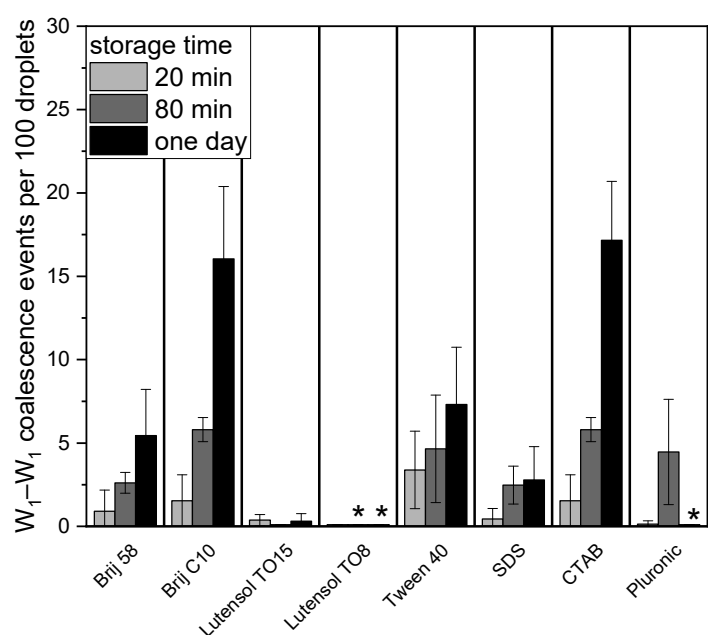


Figure 6. Number of W_1-W_1 coalescence events normalized to 100 droplets for 0.9 wt% of different hydrophilic surfactants in W_2 and 1 wt% of PGPR in O . Apart from the Lutensol TO15 types, all double emulsions had coalesced inner droplets. The bars marked with an asterisk could not be measured due to major instabilities because of $O-O$ and W_1-W_2 coalescence.

Dependent on the chemical compatibility of the surfactant and the oil phase, transport of hydrophilic surfactant molecules can be slowed down or completely prevented. For longer transport distances (1 cm) through oil, it was found in an earlier study [54] that Brij C10 and Brij 58 arrive at the inner interface within seconds, while CTAB and SDS needed more than 24 h to come to the inner interface. In the microfluidic $W_1/O/W_2$ double emulsion droplets used for these experiments, the oil layer between the W_1/O and O/W_2 interphase is only some micrometers thick. For CTAB and Brij C10 (ionic or nonionic surfactant), increased coalescence of inner droplets was already observed after 20 min. The stability trends found for the four mentioned surfactants differ slightly from the results of our earlier study on W_1-W_1 coalescence in single emulsion droplets [26], which may be explained by a different PGPR concentration (0.1 wt% vs. 1 wt%) being applied. This again points to the need for a detailed study on different surfactant concentrations in future work.

4. Discussion

Table 2 summarizes and compares the stability of each formulation against all three coalescence mechanisms. To guide the eye, good stability values are marked in green,

medium stability in yellow and poor stability is shown in red. Additionally, letters are given for groups of significant difference with ANOVA at a level of 0.05. The significance groups apply within each column.

Table 2. Evaluation of the stability against each possible coalescence paths for the combination of PGPR (1 wt%) with different hydrophilic surfactants (1 wt%). The values and groups of significant difference ($p > 0.05$) are given for the double emulsion droplets after one day of storage. The color scheme is given to guide the eye for good (green), intermediate (yellow) and poor (red) stability against coalescence, as observed in the experiments. Grey values could not be measured due to complete phase separation.

Stabilized with PGPR and	O–O Coalescence per 100 Droplets	W ₁ –W ₁ Coalescence per 100 Droplets	W ₁ –W ₂ Coalescence per 100 Droplets
Brij 58	0.7 ^a	5.4 ^a	3.0 ^a
Brij C10	5.4 ^b	16.0 ^b	67.2 ^b
Lutensol TO15	0.0 ^a	0.3 ^a	9.5 ^c
Lutensol TO8	0.6 ^a	0.0 ^a	99.0 ^d
Tween 40	0.0 ^a	7.3 ^a	8.3 ^c
SDS	0.0 ^a	2.8 ^a	6.2 ^{ac}
CTAB	1.9 ^{ab}	17.2 ^b	80.2 ^d
Pluronic	99.0 ^c	– ^c	– ^e

For any application, the coalescence mechanism the formulation stabilized poorest against is the critical one. Going over Table 2, Pluronic can be ruled out due to poor O–O coalescence stability. Brij C10 and CTAB are disadvantageous due to increased W₁–W₁ coalescence and some W₁–W₂ coalescence. Double emulsions stabilized with Lutensol TO8 collapse due to W₁–W₂ coalescence. For the applied concentrations, and in combination with MCT oil and 1 wt% PGPR as lipophilic surfactant, SDS and Brij 58 can be suggested for stable formulations. Tween 40 and Lutensol TO15 can also be considered when slow release rates and small changes in W₁ droplet size (and the consequent osmotic swelling) can be tolerated. For all surfactants, the time scales in which instabilities occur will vary strongly, dependent on the droplet sizes, while the general trend remains the same [42]. Therefore, the rather short stability of all formulations against all coalescence mechanisms is much more pronounced than is expected in industrially relevant double emulsions with much smaller droplet sizes.

For all coalescence mechanisms, examples of good, medium and poor stability were found in this work. However, mechanistic explanations of which properties of the hydrophilic surfactant lead to which (in-)stability, cannot be given. There are surfactants that show similar stabilization properties (e.g., Brij 58 ≈ SDS or Brij C10 ≈ CTAB or Lutensol TO15 ≈ Tween 40), but the hydrophilic surfactants have no similarities in chemical structure. The first two pairs are an ionic and a nonionic surfactant, the last pair is of two nonionic surfactants, but one is branched, the other linear. In contrast, the four linear alcohol ethoxylates only differ in chain length and nonetheless the stabilization of droplets differs hugely. A general trend that was observed is that a low HLB value for the hydrophilic surfactant leads to less stability against all three coalescence mechanisms. Nonetheless, we cannot suggest any surfactant attribute for the use in double emulsions in general. For such statement, a larger number of hydrophilic surfactants must be examined.

The instability of the tested polymers is contradictory to the findings in the literature [12,57]. An explanation is that the stabilizing mechanism of polymers differs from the mechanisms of interfacial active short chain surfactants. While the shorter components only build a layer at the interface, polymers do additionally stabilize droplets by enhancing the viscosity of the continuous phase. The reduction of surfactant concentration in respect to the limited interfacial area in the experiments might have a destabilizing effect on the double emulsions stabilized with polymers.

As mentioned before, a next step could lie in a detailed study of the influence of the surfactants' concentration on the three coalescence pathways. Concentration changes could

be used to counteract the instabilities observed. An assumption would be that the reduction of hydrophilic surfactant concentration might increase W_1 – W_1 and W_1 – W_2 stability, while decreasing the stability of the oil droplets [31]. Additional explanations can be found by breaking down the coalescence process into its substeps [27]. In this work, only the resulting stabilities were measured and compared. The stability of two different surfactants might, however, be the result of different stabilization processes. The dominating forces in film thinning and film rupture are completely different, e.g., for ionic and non-ionic surfactants. While some interface compositions show mostly the kinetics of film drainage, other surfactant films add a film rupture time [58]. With the method applied here, this cannot be differentiated.

5. Conclusions

The introduced analysis technique on the instabilities of double emulsion can show that different double emulsion formulations change over time by different coalescence pathways. Each of the three possible coalescence mechanisms was observed as the dominant instability mechanism in at least one formulation. Some surfactant combinations were completely stable in the examined time, some formulations showed all three coalescence pathways simultaneously. With the eight examined hydrophilic surfactants, the main coalescence path could not be linked to any detail in the chemical structure of the surfactants.

Separating the three coalescence mechanisms from each other by quantifying them with one measurement technique helps in adding a better understanding of double emulsion instabilities. The knowledge about the main failure mechanism can help in optimizing a given formulation by adjusting the surfactant concentrations or phase viscosities to slow down the specific mechanism. Finally, this study can suggest SDS and Brij 58 in combination with MCT oil and PGPR to stabilize double emulsion droplets against all three coalescence mechanisms for at least 24 h.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr11010234/s1>, Figure S1: Double emulsion stabilized with Brij C10 after 20 min storage time; Figure S2: Double emulsion stabilized with Brij C10 after 80 min storage time; Figure S3: Double emulsion stabilized with Brij C10 after one day storage time; Figure S4: Photos of double emulsion droplets directly after microfluidic break-up and after one day of storage in their respective surfactant solutions. Brij 58, Lutensol TO15, Tween 40 and SDS show stable droplets. Emulsions with Lutensol TO8 are completely empty and with Brij C10 and CTAB a lot of release of inner droplets is seen. With Pluronic stabilized oil, droplets did separate completely and the sample was taken after redispersing the oil layer.

Author Contributions: Conceptualization, N.L. and H.P.K.; methodology, N.L.; formal analysis, N.L.; investigation, N.L.; resources, H.P.K.; writing—original draft preparation, N.L.; writing—review and editing, H.P.K.; visualization, N.L.; supervision, H.P.K.; funding acquisition, H.P.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available upon request.

Acknowledgments: We acknowledge support by the KIT-Publication Fund of the Karlsruhe Institute of Technology.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Calculation of HLB values after Davies [46]:

Brij 58:	$16 * (-0.475) + 20 * 0.33 + 7 = 6.0$
Brij C10:	$16 * (-0.475) + 10 * 0.33 + 7 = 2.7$
Lutensol TO15:	$13 * (-0.475) + 15 * 0.33 + 7 = 5.8$
Lutensol TO8:	$13 * (-0.475) + 8 * 0.33 + 7 = 3.5$
Tween 40:	$16 * (-0.475) + 40 * 0.33 + 6.8 + 7 = 19.4$
SDS:	$12 * (-0.475) + 38.7 + 7 = 40$

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