colloids and interfaces

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Review

Evaluating the Stability of Double Emulsions—A Review of the Measurement Techniques for the Systematic Investigation of Instability Mechanisms

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Abstract: Double emulsions are very promising for various applications in pharmaceutics, cosmetics, and food. Despite lots of published research, only a few products have successfully been marketed due to immense stability problems. This review describes approaches on how to characterize the stability of double emulsions. The measurement methods are used to investigate the influence of the ingredients or the process on the stability, as well as of the environmental conditions during storage. The described techniques are applied either to double emulsions themselves or to model systems. The presented analysis methods are based on microscopy, rheology, light scattering, marker detection, and differential scanning calorimetry. Many methods for the characterization of double emulsions focus only on the release of the inner water phase or of a marker encapsulated therein. Analysis methods for a specific application rarely give information on the actual mechanism, leading to double emulsion breakage. In contrast, model systems such as simple emulsions, microfluidic emulsions, or single-drop experiments allow for a systematic investigation of diffusion and coalescence between the individual phases. They also give information on the order of magnitude in which they contribute to the failure of the overall system. This review gives an overview of various methods for the characterization of double emulsion stability, describing the underlying assumptions and the information gained. With this review, we intend to assist in the development of stable double emulsion-based products.

Keywords: multiple emulsion; emulsion stability; coalescence; diffusion; encapsulation efficiency

1. Introduction

Double emulsions are emulsions within emulsions, i.e., the droplets are emulsions themselves. The commonly investigated double emulsion morphology is the water-in-oil-in-water (W/O/W) type. W/O/W emulsions are water-continuous systems, containing oil droplets in which smaller water droplets are dispersed. As the inner and outer W phases typically differ in their composition, the abbreviation W1/O/W2 is used, which distinguishes between the inner (W1) and the outer (W2) aqueous phase.

With such systems, it is possible to produce fat-reduced products, e.g., salad sauces. The commonly used oil-in-water (O/W) emulsion is replaced by an equivalent W/O/W emulsion with a lower actual oil content but a similar texture perceived in the mouth [1]. W1/O/W2 emulsions also show great potential as drug delivery systems for sensitive (bio-)active ingredients. The active ingredients, such as enzymes, vitamins, or pesticides, are encapsulated within the inner water droplets (W1) and then released over time or due to a specific trigger, e.g., temperature or pH change [2,3].

The challenge in producing double emulsions is the stabilization of the two different interfaces throughout further emulsion processing and storage. Additionally, for some applications, it must be

possible to destabilize those interfaces at a certain moment, e.g., for the release of the encapsulated ingredient.

In contrast to conventional emulsions where one emulsifier is enough, double emulsions require a minimum of two emulsifiers: a lipophilic emulsifier is necessary to stabilize the inner water droplets, while a hydrophilic one is used to protect the oil droplets from coalescence [4]. Even though much is known about which emulsifiers are suitable for conventional O/W and W/O emulsions, this knowledge cannot be directly transferred to double emulsions. In double emulsions, the individual emulsions are linked via the same O-phase, meaning that emulsifiers, active ingredients, and water molecules are able to diffuse from one phase to the other and interact at the interfaces [5]. As a result, double emulsions are highly susceptible to breakdown during storage or when exposed to environmental stresses [6].

Several studies are available that focus on the comparison between emulsifier systems and emulsifying machines on the stability of double emulsions [2]. Commonly used emulsifying machines in the field of double emulsions are high-pressure homogenizers, rotor-stator-systems, membrane emulsification, and microfluidics. All of these devices can be employed at varying process conditions. For a specific system, the production conditions can be adapted to achieve the desired emulsion properties. The production of double emulsion is usually split into two steps: First, the inner emulsion is produced, and then the inner emulsion is dispersed in the outer water phase in a second step. The emulsifying machines and parameters can differ in both steps. Most of the published research shows that the performance of a double emulsion can be enhanced by adapting the process or formulation for its desired application [2].

Once produced, double emulsions are subjected to several coalescence and diffusion phenomena that consequently impact product properties, such as texture or encapsulation performance. Analyses often show how quickly the texture changes or an encapsulated active substance is lost, but cannot depict which mechanism (coalescence and/or diffusion) is responsible for it.

In this review, the different measurement techniques for analyzing instability mechanisms are discussed. Our focus is to show which boundary conditions must be applied for the respective measuring method, which learnings they offer, and how these can help in the improvement of double emulsion formulations.

2. Instability Mechanisms in Double Emulsions

2.1. Distribution of Emulsifiers and Osmotic Active Ingredients in Double Emulsions

Double emulsions are stabilized by two different surface-active ingredients: The lipophilic, oil-soluble emulsifier stabilizes the inner W1 droplets in the W1/O emulsion, while the hydrophilic emulsifier has the task of stabilizing the filled oil droplets to stop them coalescing with each other. Many combinations of hydrophilic and lipophilic emulsifiers have been studied, as summarized in several review articles [2,7,8]. Figure 1a shows the idealized distribution of the different emulsifier molecules. The lipophilic emulsifier occupies the inner interface, while the hydrophilic molecules adsorb at the outer one. Free lipophilic emulsifier molecules are dissolved in the O phase, while hydrophilic emulsifier molecules are only found in the W2 phase. Lipophilic and hydrophilic molecules do not interact, nor do they disturb each other in their action.

However, emulsifier molecules are amphiphilic, meaning that they are soluble in and distribute between both phases and interfaces. Consequently, lipophilic emulsifier molecules will also adsorb, to some extent, at the outer interface. Moreover, even if hydrophilic emulsifier molecules prefer to be dissolved in an aqueous phase or adsorb at an O/W2 interface, some of them may also diffuse through the O phase. As a result, they can adsorb to the W1/O interface and interact with the lipophilic emulsifier molecules there. The extent to which this happens will mainly depend on the solubility of the hydrophilic emulsifier in the O phase separating the interfaces from each other. Therefore, a more realistic distribution of the emulsifier molecules in a W/O/W double emulsion is depicted in

Figure 1b [9]. When different emulsifiers adsorb at an interface, they will interact with each other. Changes in the stability of the double emulsion are to be expected. The molecular structure of the emulsifiers and the oil phase influence the distribution of the emulsifiers between the phases.

Furthermore, it is common practice to add osmotic active substances to the inner water phase in order to balance the capillary pressure between the inner and the outer water phase and prevent Ostwald ripening. Over time, osmotic active substances can also distribute via diffusion or coalescence, as shown in Figure 1.

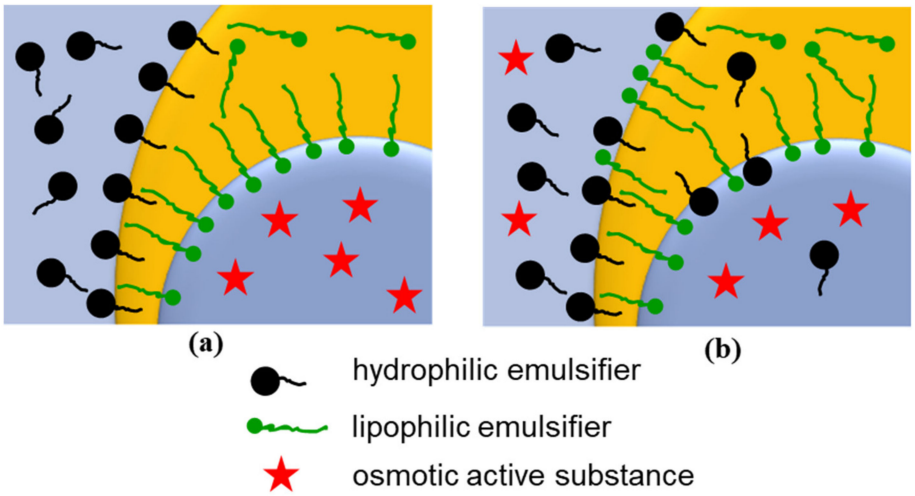

Figure 1. Schematic drawing of the composition of the interfaces in double emulsions. (**a**) Idealized distribution: each emulsifier only adsorbs at the interface it prefers, and the osmotic active substance is dissolved in the inner water phase. (**b**) Realistic distribution: the emulsifiers will distribute between all phases and interfaces and interact with each other at the interfaces; the osmotic active substance is found in both water phases.

2.2. Instabilities via Coalescence

Figure 2 outlines the irreversible instability mechanisms occurring in double emulsions: coalescence and diffusion. Other instability phenomena, like agglomeration and creaming, can accelerate these mechanisms but are themselves reversible. Therefore, these phenomena are not the focus of this review.

On the right side in Figure 2, the possible types of coalescence are shown. W1–W1 and O–O coalescence are similar to typical coalescence phenomena in single emulsions: A droplet merges with a droplet of its kind. The interfaces approaching each other are of a similar curvature, and the molecular species at the interfaces are alike. However, the actual composition of the interface is unknown in double emulsions due to the competitive adsorption of different emulsifiers [7].

W1–W1 coalescence does hardly change the behavior of the double emulsion. Although the droplet size distribution of the inner emulsion changes, the properties of the double emulsion do not, as they mainly depend on the oil droplet size distribution and volume ratio of the (filled) oil droplets. However, larger W1 droplets may behave differently in W1–W2 coalescence. Furthermore, the capillary pressure within the W1 droplets is lower when their size increases. Thus, influences on diffusion are to be expected.

In contrast to W1–W1 coalescence, O–O coalescence does change the properties of the double emulsion. First, larger droplets cream faster. Additionally, the viscosity of the double emulsion decreases when the oil droplet size increases, which further enhances creaming. In extreme cases, the double emulsion will separate into a water phase and a W/O emulsion.

The third type of coalescence shown in Figure 2, the coalescence of inner droplets (W1) with the continuous water phase (W2), is critical because of the release of the encapsulated substance (water and any dissolved active ingredient). In extreme cases, the double emulsion breaks so that only an O/W

emulsion remains. In W1–W2 coalescence, two interfaces of very different curvature and of different emulsifier composition get into contact. Based solely on the composition, it is therefore very difficult to predict whether a double emulsion is stable or not.

The reversion of W1–W2 coalescence is called spontaneous emulsification and can occur for certain emulsifiers [10]. For polyglycerol polyricinoleate (PGPR), one of the most common lipophilic emulsifiers used in double emulsion formulations, spontaneous emulsification has been reported [11]. The outer water phase is emulsified without mechanical treatment into the oil droplets, which leads to a small increase of the inner water phase. The encapsulated water volume in the oil, however, is relatively small and does not change the proportion of internal water. This mechanism can work in addition to diffusion to achieve an accelerated equalization of the pressure between W1 and W2 phase change [11,12].

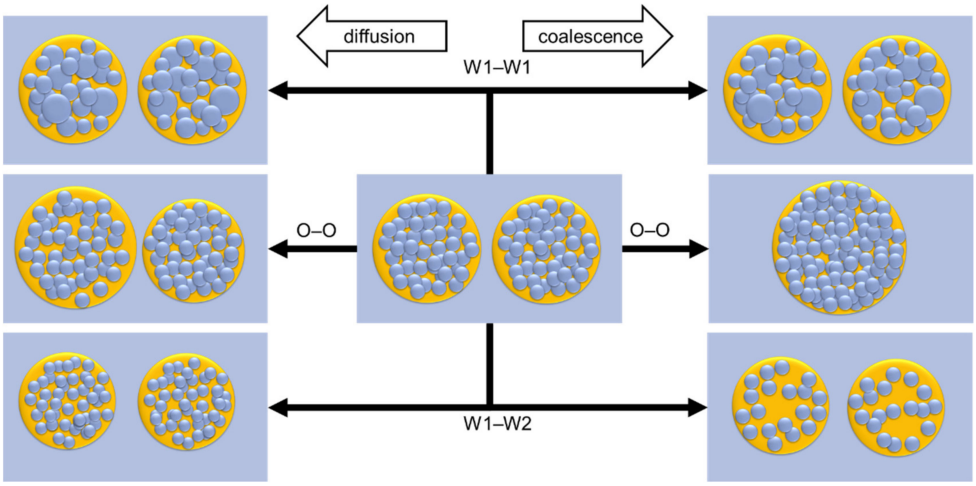

Figure 2. Mass transfer in double emulsions. In the center, the stable emulsion is shown. To the left, the changes due to diffusion are shown, and to the right, changes through coalescence are depicted. The scheme in accordance with other authors [7,13].

2.3. Instabilities via Diffusion

Figure 2 on the left side depicts mass transfer due to diffusion. The first two types are also found in single emulsions: differences in droplet size lead to differences in capillary pressure. This pressure difference is the basis for Ostwald ripening: larger droplets grow and smaller ones shrink. This phenomenon can be enhanced by certain emulsifiers or combinations thereof [14]. A common method to limit this type of diffusion is the production of monodisperse droplets, preferably by membranes or microfluidic devices [15,16]. Furthermore, the addition of osmotic active substances to the W1 phase will help as well, as diffusion between water droplets will end once osmotic pressure differences balance capillary pressure differences.

The diffusion phenomenon specific to double emulsions is the diffusion from the inner water phase to the outer water phase (W1–W2). Water transfer between W1 and W2 phase change the size of the oil droplets and the volume fraction of the different phases at the same time. Depending on the osmotic pressure difference between W1 and W2, W1 droplets will shrink or swell [2,17]. In consequence, the size of the oil droplets also changes. W1 shrinkage will reduce the performance of fat-reduced products and decrease double emulsion viscosity. When W1 droplets grow, the simultaneous loss of the continuous W2 phase and the consequent growth of the oil droplets increases the viscosity of the double emulsion drastically (osmotic swelling) [18].

The driving force is the pressure inside the inner water droplet. It is the sum of the capillary pressure of both curved interfaces between W2 and W1 [19]. The capillary pressure of the outer water phase equals zero. In double emulsions, it is possible to formulate inner and outer water phase differently. This way, the osmotic pressure inside the W1 droplets can be tailored to counterbalance the

capillary pressure. To do this, the interfacial tension and droplet size distributions must be known [2]. It is also reported, that Ostwald ripening is reduced by converting W1 droplets into soft solid-like particles through gelation [2,7,20].

2.4. Interaction of Instabilities

Coalescence and diffusion phenomena change the emulsion structure, texture, and encapsulation efficiency: the encapsulation efficiency usually decreases, the viscosity changes, and phases separate. Therefore, measurement methods that are based on the encapsulation efficiency, on viscosity changes, or phase separation only monitor the change of these emulsion properties over time or after a certain treatment. However, they cannot tell the mechanism of instability responsible for the observed changes.

In addition, if double emulsion formulations fail, they do so due to several mechanisms at the same time. Interactions between coalescence and diffusion are described in literature for several formulations: when the inner water droplets coalesce, and thus increase their size, release is increased as well [21]. Khadem et al. [22] reported the spontaneous breakdown of the double emulsion structure after a certain degree of osmotic swelling of the inner water droplets. These examples illustrate that the initial instability mechanism can be different from the observed result.

Finding suitable countermeasures for the instability of the emulsion is therefore difficult. This is why so many studies on double emulsion report changes in the emulsion composition and/or production method and the resulting effects on product properties. The reviews summarize all these findings in tabular form and try to draw conclusions [2,8,23].

3. Measuring Instability in Double Emulsions

3.1. Microscopy

Optical methods can give an impression of changes in droplet size or filling degree. As the amount of droplets evaluated within one image is limited, effects are evaluated on a qualitative level. However, optical methods also give highly detailed information, which allows for an interpretation of the underlying mechanisms. Figure 3 shows examples of microscopic images. On the left side, highly filled oil droplets are shown. The oil droplets are deformed by the glass cover slide, which allows for visualizing the inner water droplets. Black areas in the oil droplets indicate other inner water droplets out of focus levels. The right picture shows a double emulsion droplet with only a few remaining inner droplets. In literature studies, microscopy is applied to prove the existence of double emulsion structures [24], show the qualitative loss of the inner water phase [25,26], visualize increasing droplet sizes of oil and inner water [17,25,27], make general comparisons of different double emulsions [28–30], and track the loss of single droplets over time [31].

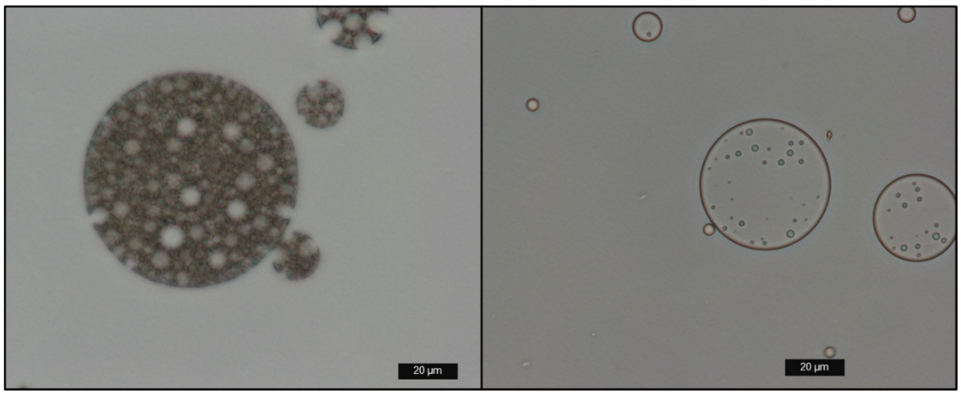

Figure 3. Examples of optical microscope images of double emulsions. Left: stable double emulsion droplets, which contain many inner droplets, resulting in dark droplets. Right: most of the inner droplets are lost due to coalescence or diffusion. Black bar: 20 µm.

Quantifying effects with microscopy is often challenging. Quantitative size determination requires a minimum of 2000 droplets, with 9000 being recommended for statistically reliable values [32]. A loss of internal water droplets is easily recognizable in a microscopic image. However, it is practically impossible to tell whether diffusion or coalescence was the reason for the loss. In order to be able to make statements about this, the filled drop must be observed continuously over a long period of time. Additionally, the deformation of the relatively big oil droplets by the cover slide makes droplet size determination difficult [33].

Microscopy is also limited to size scales that can be accessed optically. Therefore, only double emulsions with oil droplets in the size range above a few µm, better a few tens of µm, can be analyzed by light microscopy. Internal water droplets below one micron can only be detected by the black coloration of the oil droplets. The accessible size range can slightly be extended downwards by using fluorescent dyes and confocal laser scanning microscopy (CLSM). This also allows for a precise differentiation between lipophilic and hydrophilic phases, as shown in Figure 4 [34]. Diffusion was also observed by CLSM videos [34–37].

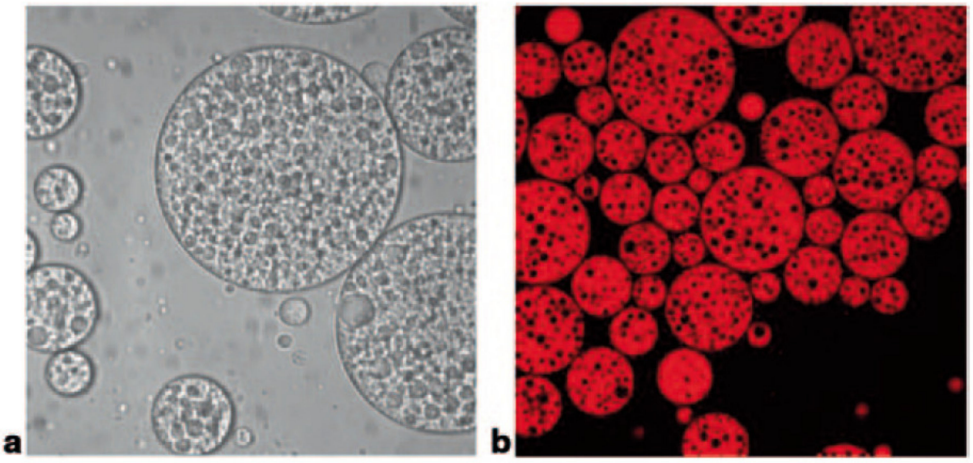

Figure 4. Comparison of a light microscope image (**a**) and a confocal laser scanning microscope (CLSM) image (**b**) of the same double emulsion. The contrast in the CLSM image was achieved by the addition of Nile Red in the oil phase. Figure taken from Bernewitz et al. [34].

3.2. Droplet Size Distribution of the Inner Emulsion

The droplet size distributions (DSD) of double emulsions can be measured with the same methods available for single emulsions, e.g., dynamic and static light scattering and image analysis. The droplet size distribution of the inner emulsion is usually measured before the second emulsification step, i.e., in the W1/O single emulsion. After the second emulsification step, the inner droplets are not accessible anymore for common measurement techniques [27]. Several studies showed that inner droplets are lost during the second emulsification step. The extent of the loss depends on the energy input [5,38,39], the emulsifier system [40], and the droplet size [40,41]. Consequently, it cannot be assumed that the droplet size distribution of the inner water droplets after the second emulsification step is the same as before.

Schuster et al. [32] described confocal laser scanning microscopy as a technique to measure the DSD of the inner water droplets after the production of the double emulsion and over time. Similar to the measurements with a marker substance, a dye is necessary, which is potentially changing the emulsion behavior [32,42].

Pulsed field gradient nuclear magnetic resonance (PFG-NMR) can also be used to determine the internal droplet size distributions. The differences in the diffusion coefficients of inner and outer water are used to distinguish between the phases and to determine the DSD of the inner phase. Several studies showed good accordance with CLSM [43,44] and laser diffraction [45]. The evaluation of the

NMR spectrum is based on the assumption that drops are spherical. For double emulsions with high filling degrees and deformed droplets, deviations may occur [45]. NMR on double emulsions is also limited to W1 droplet sizes with a minimum of 2 µm [46].

3.3. Droplet Size Distribution of the Outer Emulsion

The size distribution of the oil droplets is easier to measure, but must be interpreted with caution. As a consequence of the loss of the inner water phase during the second emulsification step, the oil droplet size is also influenced. By changing the phase distribution, the viscosity ratio between the disperse W1/O phase and the continuous W2 phase changes. This viscosity ratio has an influence on the oil droplet break-up [31,47]. Therefore, the oil droplet size distribution directly after the break-up not only depends on the stability of the double emulsion but also on processing conditions.

The change of the oil droplet size over time can indicate oil droplet growth due to coalescence [48] or osmotic swelling [17], or droplet shrinkage due to a loss of inner water [17]. The change rates can be linked to the stability of the double emulsion system. However, one cannot deduce whether diffusion or coalescence is the reason for a change in oil droplet size. Figure 5 shows the development of the DSD of a double emulsion over time. The inner water phase has a high osmotic pressure and osmotic swelling is expected. Whether oil drops coalesce at the same time and contribute to the drop growth measured cannot be deduced from these data.

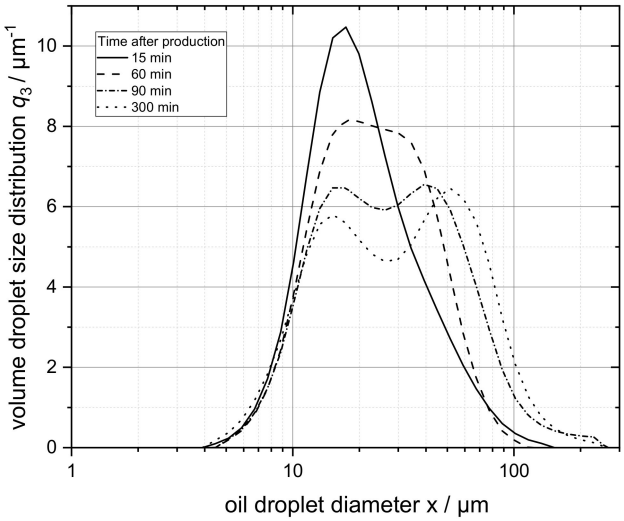

Figure 5. Oil droplet size distributions (DSD) of a double emulsion over time. Inner water/oil/outer water (W1/O/W2) phase composition: W1: aqueous 0.35 wt % NaCl solution; O: canola oil with polyglycerol polyricinoleate (PGPR); W2: aqueous polyvinyl alcohol solution. Droplet sizes are measured with static laser diffraction. Droplet growth may have occurred due to coalescence or osmotic swelling.

3.4. Rheology

The rheological behavior of an emulsion depends on its internal structure. Mostly the viscosity is measured, which is closely connected to the amount of disperse phase and its DSD [49]. Changes in viscosity can be linked to changes in the emulsion structure, which is also true for double emulsions [50].

With different models (e.g., the Krieger–Dougherty equation), the viscosity of an emulsion is described as a function of its disperse phase fraction [51]. For double emulsions, the encapsulation efficiency can be calculated from the viscosity data [52]. The disperse phase ratio to viscosity relation is also used to show osmotic swelling [18]. An example for the viscosity effects is shown in Figure 6: double emulsions were produced with different amounts of hypotonic water encapsulated in the oil droplets but with otherwise comparable formulation. The high osmotic pressure inside the inner water

drops leads to swelling of the droplets and to an increase of the disperse phase fraction. The more water encapsulated at the beginning, the more pronounced is the change of viscosity.

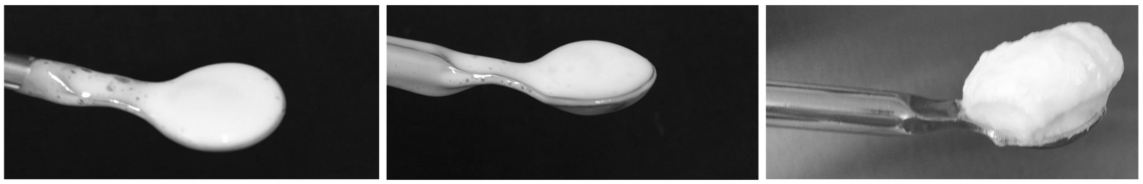

Figure 6. Double emulsions with comparable formulation but different initial W1 fractions. The W1 phase is hypotonic, leading to osmotic swelling of the W1 droplets. The effect of osmotic swelling on double emulsion viscosity is more pronounced (higher viscosity) when the initial W1 fraction is higher. W1/O/W2 phase composition: W1: aqueous 0.35 wt % NaCl solution; O: canola oil with PGPR; W2: aqueous polyvinyl alcohol solution. Left to right: 30%, 50%, and 70% of initial inner water phase.

3.5. Encapsulation Efficiency via Release of Marker Substances

The detection of released marker substances is a widespread method for determining the stability of double emulsions. It measures the property for which double emulsions are most commonly produced: the encapsulation and targeted release of active ingredients [23,53]. For analysis, marker substances are encapsulated that are easy to analyze. Lists with examples of suitable substances and their corresponding detection method can be found in Lamba et al. [8] and Dickinson and Muschiolik [2]. Detection methods range from conductivity (e.g., [38]) over spectroscopy (e.g., [26]) to more complex methods like NMR spectroscopy (e.g., [46]).

By comparing the release of different marker substances from the same formulation, it becomes clear that the release does not depend solely on the double emulsion stability but also on the molecular structure of the marker substance. In conductivity measurements, the type of ion influences the kinetics of release, e.g., iodine ions are released almost twice as fast as bromine, fluorine, or chlorine ions [30]. The encapsulated protein (e.g., bovine serum albumin) is released much slower than encapsulated fluorescein (FITC) [28]. Methylene Blue can be better encapsulated than Vitamin B12 [54]. Figure 7 shows an example of the effect of the selected marker on the release. Brilliant Blue is released to a greater extent than black carrot concentrate from the same double emulsion for all three different manufacturing parameters tested.

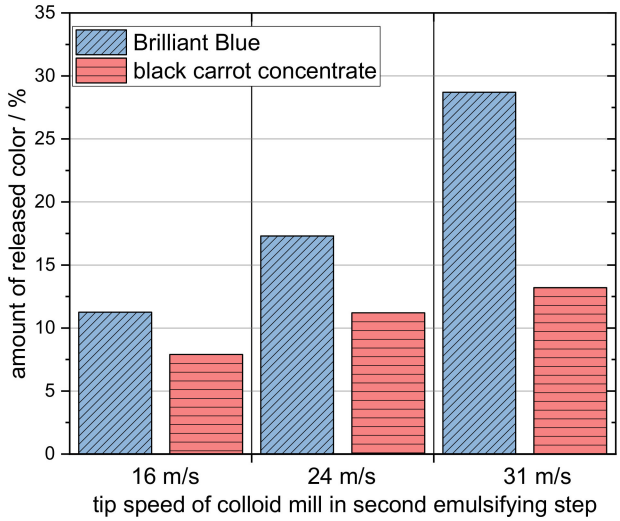

Figure 7. Relative release of different markers from a double emulsion formulation. W1/O/W2 phase composition: W1: aqueous 0.35 wt % NaCl solution; O: canola oil with PGPR; W2: aqueous polyvinyl alcohol solution. The emulsions were prepared with three different shear rates in the second emulsifying step to achieve different releases.

The observed differences arise either from different diffusion rates of the marker substances or from interactions between the marker and the emulsifiers. Markers are transported either as single molecules or in inverse micelles [43]. It has also been shown that ions, for example, change the stabilization by PGPR and thus the release of encapsulated substances [55].

To make general statements on stability, a variety of substances should be tested and compared. Encapsulation efficiency values can usually be compared qualitatively as long as the same marker substance is used.

3.6. Encapsulation Efficiency via Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) allows for the marker-free determination of encapsulation efficiency. This measurement method makes use of the fact that water encapsulated in very small droplets (W1) freezes at significantly lower temperatures than bulk water (W2). It was first described by Potier et al. [56] and later applied by several research groups [38,47,57–59]. Figure 8 shows a DSC trace obtained from cooling and heating a double emulsion. The inner and outer water phases freeze at different temperatures. The peaks from left to right correspond to O, W1, and W2. The water distribution can be determined from the areas under the respective peaks. Results obtained by this method match the encapsulation efficiencies determined by viscosity measurements and marker methods [38].

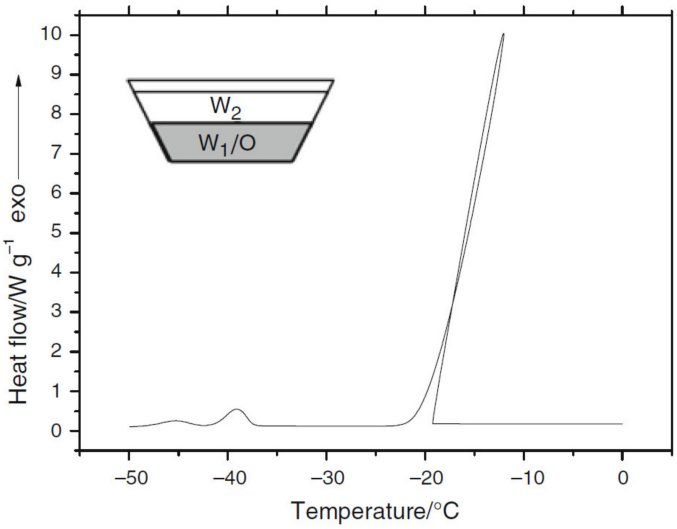

Figure 8. Example of a differential scanning calorimetry (DSC) plot for double emulsions. The encapsulation efficiency can be calculated from the areas under the peaks. The Figure was taken from Schuch et al. [58].

Neumann et al. [60], however, showed the limitations of this measurement technique. If the emulsion system is not freeze resistant, the measurement itself can lead to a massive loss of the inner water phase, resulting in incorrect values for encapsulation efficiency. This effect must be excluded before performing DSC measurements.

3.7. Encapsulation Efficiency via Creaming of Oil Droplets

The creaming rate of droplets can also be used to determine the encapsulation efficiency. The creaming velocity of droplets correlates with their density, and the density of the droplets depends directly on the filling degree of the drops [61].

Not only the creaming velocity, but also the phase fraction after creaming can give information on the stability of the double emulsion. When inner water is released, the volume of the oil droplets is also reduced while the amount of outer water phase increases. An increased volume fraction of the outer water phase at the bottom of the analysis vessel shows a release of the inner water phase [50,62].

Figure 9 shows the results of accelerated creaming in an analytical centrifuge. Different disperse phase fractions lead to a visible shift in the phase boundary. This allows differences in phase distribution to be measured quickly and easily.

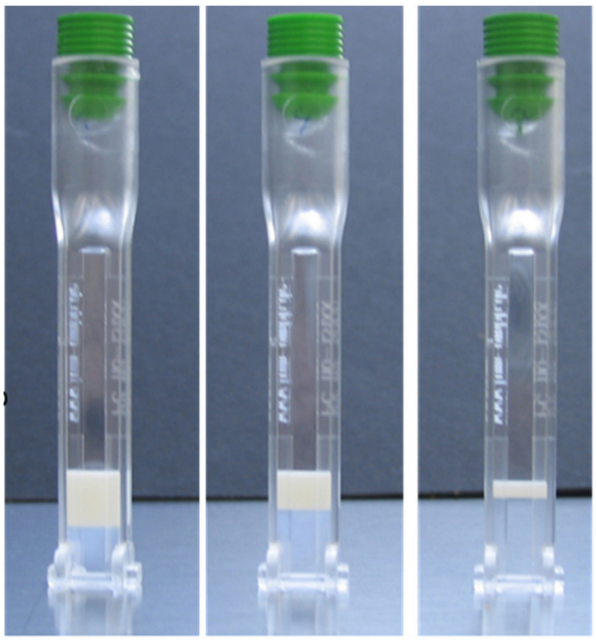

Figure 9. Double emulsions after centrifugation with an analytic centrifuge, LumiSizer (LUM GmbH, Berlin). Different amounts of transparent phase show different amounts of outer water phase. W1/O/W2 phases consist of aqueous 0.35 wt % NaCl solution, canola oil with PGPR and of aqueous whey protein isolate, respectively.

4. Measurements on Models of Double Emulsions

Reducing double emulsions to models can simplify the measurement and can help detect the underlying instability mechanisms. There are different ways to simplify complex double emulsions: the reduction of phases, increase of the size of the droplets, thus reducing the curvature of the interfaces, and the observation of single droplets. The measurement methods stay the same: optical measurements, the measurement of droplet size, marker concentration, creaming rate, or rheological characteristic values (e.g., viscosity).

4.1. Reduction of Phases—Single Emulsion Experiments

The double emulsion structure is reduced to single emulsions each containing the relevant components of the multiple emulsion [9,55,63], as shown in Figure 10. Thus, the inner emulsion, W1/O (a and b), and the outer O/W2 emulsion (c) are investigated separately. It is important to keep the stability-relevant parameters (droplet size, emulsifier concentration) at values comparable to the original double emulsion [63]. In a two-step preparation process, the inner W1/O emulsion can be analyzed prior to the second emulsification step. In doing so, the influence of the hydrophilic emulsifier on the stability of the inner emulsion is neglected. The inner emulsion can be examined to detect, e.g., interactions between osmotic active substances and the lipophilic emulsifier [55] or the influence of the encapsulated substance on the stability of the inner emulsion [48] (case a).

Case (b) shows that hydrophilic emulsifier molecules have diffused through the O phase to the W2 phase. It is achieved by adding hydrophilic emulsifier molecules to the W1 phase before the first emulsification step. The expected concentration of the hydrophilic emulsifier in the W1 phase must first be calculated from the distribution equilibrium of the hydrophilic emulsifier in the phases of the corresponding double emulsion.

Case (c) allows for the measurement of the effect of emulsifier interactions at the outer interface. The lipophilic emulsifier has to be dissolved in the oil phase prior to emulsifying it in the W2 phase containing the hydrophilic emulsifier. If the interactions of the two emulsifiers at the O–W interface destabilize the single emulsion, then there is a change in the O/W droplet size distribution. Neumann et al. [63] could prove that the corresponding double emulsion will then also be destabilized. Producing single emulsions is much faster and easier than producing double emulsions. Changes in droplet size distributions are safer and easier to measure than experiments on double emulsions. Therefore, this procedure is a quick method for selecting emulsifier systems suitable for double emulsions.

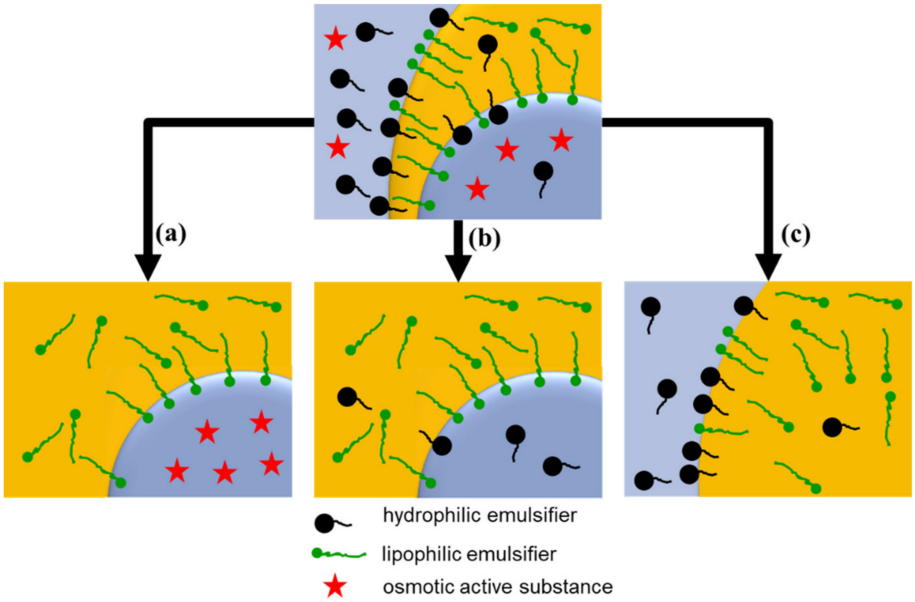

Figure 10. Possible simplifications of a double emulsion to measure instability mechanisms. Simplifications to investigate the inner emulsion (**a**,**b**), and the outer emulsion (**c**). Models proposed by Neumann et al. [63].

4.2. Double Emulsion Droplets of Defined Size—Microfluidic Double Emulsions

Microfluidic devices allow for producing double emulsions of defined droplet size und filling degree [64–66]. Either symmetrical glass capillaries (e.g., [67–71]) or Polydimethylsiloxane chips (e.g., [64,72,73]) have been proposed for this task. In contrast to double emulsions produced by typical industrial processing machines, oil droplets contain a smaller number of inner water droplets. The number is limited to less than ten inner drops when monodisperse droplets are needed [70]. Most microfluidic double emulsions however, are so-called core-shell droplets. One water droplet is inside each oil droplet, resulting in a water droplet surrounded by a layer of oil. The amount of water in the oil droplet can be adjusted up to over 90% of inner water phase [74].

Due to the small number of inner droplets, the oil droplets are transparent and therefore accessible to optical measurements. Diffusion and coalescence can be observed microscopically, as shown in Figure 11. The ability to adjust the inner and outer droplet sizes precisely also allows for systematic investigations of the influence of size on the stability of the double emulsion. Villa et al. [21] were able to detect that a reduced encapsulation efficiency is caused by W1–W1 coalescence, which then leads to W1–W2 coalescence and thus the release of the encapsulated substance.

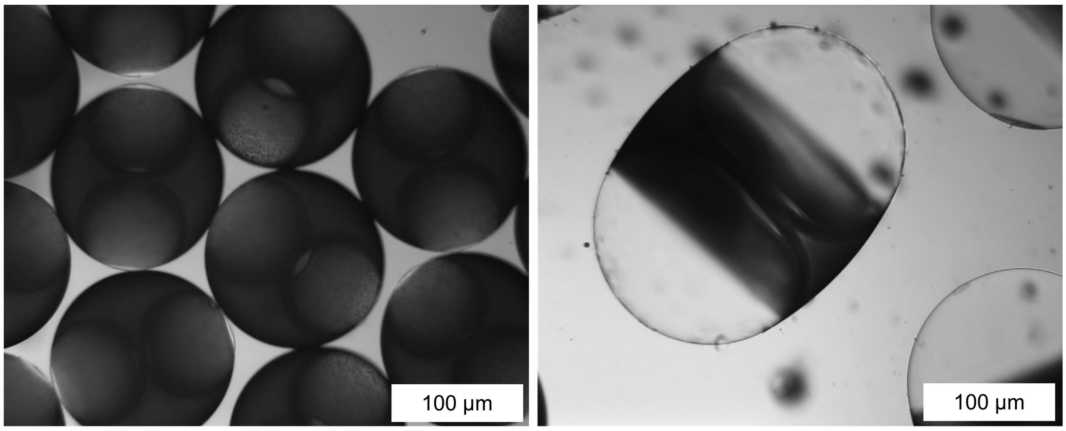

Figure 11. The same microfluidic double emulsion with two inner water droplets directly after production (left) and after 5 days (right). W1/O/W2 phases consist of an aqueous 0.35 wt % NaCl solution, canola oil with PGPR, and of aqueous polyvinyl alcohol solution, respectively. After 5 days, water droplets are swollen due to osmotic swelling and the oil droplets deform. White bar: 100 µm.

The influence of different emulsifiers is also easy to investigate with this model system. Sanders et al. [75] observed the influence of the hydrophilic emulsifier on the coalescence of inner droplets with the outer phase. Microfluidic double emulsions are also often used to investigate osmotic swelling. The challenge of the capillary pressure distribution resulting from inner droplet being polydisperse [76] is significantly reduced in microfluidic emulsions, as all droplets are of the same size [19,77]. The number of analyzed droplets needed for the significant determination of changes is also reduced by the monodispersity of the droplets. Guan et al. [70] and Hou et al. [14] could deduce from their experiments that osmotic swelling causes the inner water droplets to be pushed against the outer interface, inducing W1–W1 coalescence (see Figure 11 on the right). In addition, to elucidate instability mechanisms, the kinetics of coalescence can also be very precisely determined in an optical accessible microfluidic device.

Optical accessible microfluidic devices also allow for investigating other interesting effects in double emulsions: Adams et al. [78] observed the coalescence of inner droplets triggered by increased temperature. Bahtz et al. [11] described the kinetics of diffusion, including spontaneous emulsification as a transport mechanism.

4.3. Single Droplet Experiments

Two other simplifications of double emulsions are i) two single droplets in contact, or ii) one single droplet in contact with an interface. A lot of research has been conducted on coalescence between two droplets [79–83]. This model resembles either W1–W1 coalescence or O–O coalescence. When one single droplet is brought into contact with a quasi-planar interface, the coalescence of this droplet with a bulk phase can be observed [84–87].

The experimental set-up for observing the coalescence of single droplets with a planar interface comprises droplets that are attached to a capillary and brought into contact with the interface mechanically [76,88]. However, it is also possible to let the droplets detach from the capillary and contact under gravity [60,89–92]. In these experiments, the deformation of the droplet while coalescing with the interface can be observed. Depending on the deformation of the observed droplet, daughter droplets are formed and remain in the O-phase [85,86]. Furthermore, the coalescence time of a droplet with a planar interface can be analyzed [60,76,88,93,94]. It is defined as the time between the contact of the interfaces and the complete disappearance of the droplet. The sizes of the droplets investigated in these single droplet experiments are between 2 µm and 2 mm, which is, in many cases, bigger than the typical inner water droplet in a double emulsion. It is also known that the coalescence time at the interface changes depending on the size of the droplet [84,86,92–94]. Nonetheless, the influence of

different emulsifier concentrations [88] or combinations [9,60] on the coalescence time can be evaluated provided that droplets of the same size are used. These experiments can be conducted with small effort on a wide variety of emulsifier combinations and can be used for searching promising emulsifiers. They can also be used to determine negative interactions between lipophilic and hydrophilic emulsifiers, as shown by [9,60,76]. Figure 12 shows an exemplary measurement of coalescence times as a function of the encapsulated substance. Drops with dissolved pectins sediment, through an oil-PGPR solution, coalesce to an interface stabilized with Lutensol. The time between contact and coalescence was measured. The values indicate that pectinic acid is easier to encapsulate in a double emulsion than citrus pectin.

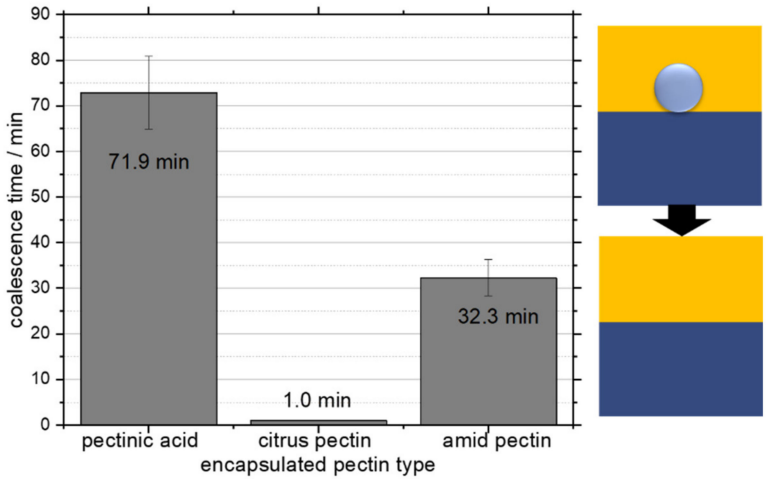

Figure 12. Coalescence time depends on the molecular structure of the encapsulated pectin. The interfaces are stabilized with Lutensol TO8 (O/W2) and PGPR (W1/O). The mean values of eight single 2 µL drops measured in succession are given.

Diffusional processes can also be observed via single droplet experiments [76]. In this case, the volume change of a W1 droplet in a defined distance to another drop or the O/W2 interface is monitored over time. Figure 13 shows an example of the volume loss of a W1 droplet at a defined distance from an O/W2 interface. The addition of emulsifiers changes both the interfacial properties and the osmotic pressure in the outer phase. This results in different shrinkage rates of the W1 droplet.

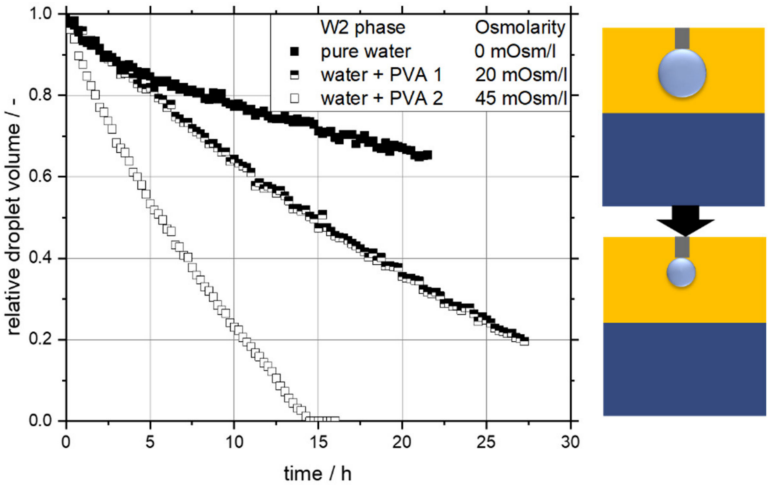

Figure 13. Shrinkage of a W1 droplet by diffusion. In the outer water phase, different polyvinyl alcohols (PVA) were added as emulsifiers, changing both the osmotic pressure and the interfacial properties. The initial drop volume was 2 µL.

5. Conclusions and Outlook

Double emulsions are very promising systems for fat-reduction or for encapsulating sensitive ingredients. Despite lots of research, only a few products have been successfully marketed to date. The underlying reason is the complexity of the double emulsion structure. Several interfaces are situated close to each other, and several emulsifiers are required. They can interact at the interfaces destabilizing them. Not only the stability during the application but also the stability during analysis are more challenging when applied to double emulsions compared to single emulsion examination.

A large number of investigations concentrate on the effect of the double emulsion formulation or its production on application properties, such as the release of an encapsulated ingredient. Unfortunately, this type of study design does not offer information on the mechanisms leading to ingredient release. However, a deeper understanding of the failure mechanism is the first step towards improving the stability of double emulsions.

We therefore summarized the measurement techniques available for the investigation of double emulsions, highlighting which information each method provides. Techniques often applied to double emulsions, such as the measurement of DSD, of viscosity, or of the release of marker substances over time, do not offer detailed mechanistic information. In contrast, the use of model experiments opens promising opportunities for mechanistic studies. Optical accessible microfluidic devices or single droplet experiments give information on the type of failure mechanism and its kinetics. They enable systematic studies on the influences of emulsifier combinations and concentrations, or of droplet sizes and filling degrees.

Only the combination of several measurement methods will give a detailed mechanistic insight into the stability of double emulsions and show possibilities for overcoming today's instability challenges. The future task will be to transfer the knowledge found in model systems back to double emulsion formulations.

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