Regular Article

Microfluidic production of size-tunable hexadecane-in-water emulsions: Effect of droplet size on destabilization of two-dimensional emulsions due to partial coalescence

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Abstract

Hypothesis: Despite numerous studies, the mechanism of destabilization of oil-in-water emulsions during cooling-heating cycles is unclear due to indirect measurements and lack of direct control over the droplet size. It is hypothesized that emulsions with a smaller droplet size are more resistant to destabilization than emulsions containing larger droplets since the probability of initiating partial coalescence and forming large-scale aggregates is lower for small droplets.

Experiments: Monodisperse hexadecane-in-water emulsions with controlled droplet sizes were produced using a microfluidic valve-based flow-focusing device and varying the system parameters. A unique approach was developed to create a two-dimensional (2D) array of droplets enabling visualization of the destabilization process due to temperature cycling. The influence of droplet size on partial coalescence and destabilization was investigated.

Findings: In the 2D emulsion, destabilization proceeds through a combination of spontaneous coalescence events that yield small-scale structures followed by formation of large-scale structures by coalescence propagation. We find that emulsion destabilization increases with droplet size. Quantifying the frequency of n-body coalescence events reveals that in emulsions with small droplets coalescence propagation is hindered. Phenomena involving restructuring, growth and cross-linking of droplet aggregates are identified as the key features of the emulsion destabilization mechanism.

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1. Introduction

Oil-in-water emulsions form the basis of numerous manufactured products such as pharmaceuticals, foods, agro-chemicals,
Various geometries, such as monodisperse droplets [26,28]. In this geometry, the droplet size fluids flowing perpendicular to each other leads to the formation of [27], have been designed for producing monodisperse microfluidic 10–15% [16], which can preclude detailed understanding of the direct control over the size of the individual droplets. The lowest droplet size[16,24].

Commercial emulsion-based products often contain a mixture of lipids [12], which makes it difficult to understand the contribution of the individual lipids to the physicochemical properties of the emulsions. To address this, model emulsions with well-defined size and composition have been widely used [13,14]. In this regard, n-alkanes have been great candidates to produce model oil-in-water emulsions. Although the chemical structure of n-alkanes is simple, they form the basis of many derivative molecules, thus making them a useful model system [15]. Among n-alkanes, hexadecane, with a melting point of 18°C [16], is a liquid at room temperature and is easy to handle for emulsification. Therefore, during the past decades, hexadecane—in-water (HiW) emulsions have been employed as a model system for emulsion studies. Some of the key phenomena studied in HiW emulsions include creaming [7], coalescence [17], Ostwald ripening [18], rheology [7], nucleation [16,19], assembly of droplet structures [20], and self-shaping [21].

Most studies employing hexadecane as the dispersed phase apply homogenization and fractionation techniques [22,23] to make the emulsions. Homogenization involves generating a polydisperse emulsion with a high-pressure valve homogenizer. Consequently, fractionation is induced by depletion flocculation to obtain relatively monodisperse emulsions. Depending on the amount of surfactant added, droplets larger than a critical size will flocculate, leaving the smaller droplets intact. By gravitational separation of the flocculated droplets and repeating this procedure several times, emulsions with different mean droplet sizes have been obtained [7]. Besides being tedious, these methods offer no direct control over the size of the individual droplets. The lowest polydispersity index (PDI) reported using such techniques has been 10–15% [16], which can preclude detailed understanding of the physicochemical phenomena in emulsions that are sensitive to droplet size [16,24].

To generate emulsions of controlled and narrow size distribution (PDI < 5%), microfluidics [25,26] has shown great promise. Various geometries, such as T-junction [17] and flow-focusing [27], have been designed for producing monodisperse microfluidic emulsions. In a T-junction device, the interaction of two immiscible fluids flowing perpendicular to each other leads to the formation of monodisperse droplets [26,28]. In this geometry, the droplet size can be controlled by adjusting the flow rate ratio of the two fluids [29–31]. However, changes in the flow rate may lead to slow response time and alter the device performance [32]. Flow-focusing devices (FFD) also offer an efficient way to produce monodisperse emulsions [33]. The droplet size in the FFD, when operated in the dripping regime [33], is proportional to the width of the drop formation orifice [34] and does not depend significantly on the flow rate ratio [33]. As a result, it is difficult to fine-tune the droplet size over a wide range in an FFD device without changing the orifice width. Nevertheless, studies have made HiW emulsions using these techniques [17,35].

Recently, to dynamically control the droplet size with a fast response time, Abate et al. implemented a membrane valve adjacent to the orifice in the FFD, which can be pressurized to deform the orifice walls and therefore change the orifice size [32]. They tested this system by generating monodisperse water-in-oil emulsions and showed that the droplet size could be tuned from 7 to 200 µm. They found a nonlinear relation between the droplet size and orifice width which they attributed to the increase in the local velocity due to the reduction in the orifice width. To date, such a flexible and robust approach has not been applied to the generation of HiW emulsions. Since these emulsions are widely studied as a model system, it is essential to identify the operating conditions for generating monodisperse HiW emulsions in a valve-based flow-focusing device (V-FFD). Knowledge from such an investigation will enable widespread use of monodisperse HiW emulsions for fundamental studies in emulsion-science.

Monodisperse microfluidic emulsions could particularly provide valuable insights into the stability of emulsions. Emulsions could break down over time through different processes such as creaming, flocculation, coalescence and Ostwald ripening [7,18]. Phase transition could also affect the stability of the emulsions due to partial coalescence, where two or more partially crystalline droplets merge, forming non-spherical aggregates [36–39]. The non-spherical shape is maintained when the elastic stresses due to the crystalline network, dominate the interfacial stresses [40]. Studies have shown that partial coalescence-induced destabilization of emulsions during cooling-heating cycles depends on oil type, volume fraction, droplet size, surfactant type, aqueous phase composition, shear rate, and cooling rate [10,36–39,41–44]. These studies used polydisperse emulsions made through homogenization and studied emulsion destabilization using indirect methods such as differential scanning calorimetry and rheometry.

More recently, using micromanipulation techniques and direct observations, Spicer and co-workers studied the stability of partially coalesced doublet and triplet structures. For doublets, they showed that the stability depends on the competition between elastic and interfacial stresses, which can be controlled by the volume fraction of crystalline content in the droplets [40]. In case of triplets, they found that the droplets can restructure into a more compact packing arrangement without fully coalescing [45]. This restructuring was shown to occur due to the expansion of meniscus that connects the droplets, resulting in the minimization of the surface energy of the triplet. It remains to be ascertained how these important insights play out when studying larger populations of droplets that model real emulsions.

The goal of this work is two-fold. First, we use the V-FFD to identify the flow and valve-actuation conditions that produce monodisperse HiW emulsions of tunable size. Second, we employ the microfluidic HiW emulsions and use microscopy to visualize and directly measure the in situ destabilization of a two-dimensional (2D) array of droplets during a cooling-heating cycle. Subsequently, we demonstrate the impact of droplet size on destabilization of the 2D emulsion due to partial coalescence. To the best of our knowledge, visualization of partial coalescence using monodisperse emulsions have not been conducted to date, since it is difficult to achieve with traditional methods.

2. Materials and methods

2.1. Design and fabrication of microfluidic devices

For this study, two microfluidic devices were fabricated. One was a valve-based flow-focusing device (V-FFD) (Fig. 1a) for droplet generation, and the other was a long serpentine channel (Fig. 1b) to store the droplets. Based on the design of Abate et al. [32], the V-FFD had a flow-focusing junction with a membrane valve on both sides of the orifice, as shown in Fig. 1a. When air pressure was applied to the membrane valves, the width of the flow-focusing orifice decreased, thereby reducing the size of
generated droplets (See Supplementary Video 1). The orifice width and height of the V-FFD were 39 ± 1 μm and 58 ± 2 μm, respectively. The valve channels had a width of 22 ± 1 μm, and the wall thickness between the membrane valves and the flow focusing orifice was 17 ± 1 μm. A 30 cm long serpentine storage device with a width of 472 ± 12 μm and a height of 98 ± 2 μm was designed to store and immobilize the HiW emulsions.

Masters for the droplet generation and the storage devices were fabricated using a negative photoresist (SU-8 2050, MicroChem) with standard photolithographic methods [46]. The replicas of the V-FFD devices were cast in poly-dimethyl-siloxane (PDMS, Sylgard 184, Dow Corning) using soft-lithography [46]. To make the membrane valves flexible in response to air pressure, a 12:1 ratio of monomer to crosslinker was used [32], and the PDMS was cured at 65 °C for two hours. After fluidic ports were made in the PDMS replicas with a 1 mm diameter biopsy puncher (Miltex), they were exposed to oxygen plasma for one minute (Harrick Plasma Cleaner) and immediately bonded to a glass slide.

Droplet storage devices were fabricated using Norland Optical Adhesive 81 (NOA81, Norland Products) [47]. First, NOA81 was poured onto the SU-8 master and cured in three steps of 10 s, under UV radiation (Loctite 7411-S UV curing system). The NOA81 layer was removed from the SU-8 mold, and the fluidic and temperature measurement ports were made using a 1.5 mm biopsy punch (Miltex). The NOA81 chip was cleaned with isopropanol and air dried. Then, a glass slide was covered with a thin layer of NOA81 and was exposed to UV for 15 s. The coated glass slide and the NOA81 chip were bonded after 60 s oxygen plasma cleaning. The device was exposed to UV light for 20 s and heat-treated at 65 °C for 15 min to ensure complete and permanent sealing. Tygon tubing (Cole Parmer) was glued to fluidic and temperature measurement ports (for insertion of thermocouples) with 5-minute epoxy (Devcon).

2.2. Droplet generation

The HiW emulsion droplets were generated using deionized water containing 2 wt% of sodium dodecyl sulfate (SDS, Sigma Aldrich) and hexadecane (Sigma Aldrich, purity > 99%) as the continuous and dispersed phases, respectively. All reagents were used
2.3. Droplet storage and temperature cycling

A long serpentine channel was used for the storage and immobilization of 2D droplet arrays (Fig. 1b) [48]. The emulsion collected in the Tygon tubing at room temperature was gently injected into the storage channel using a syringe filled with SDS solution. The droplet diameter did not change during storage and with a gentle injection of the emulsion into the storage device. Consequently, the Tygon tubing was detached from the storage device, which allowed the droplets to slow down and become immobilized. The storage device was then sealed by blocking the ports with metal pins, which reduced the motion of the emulsions during the experiments. We find that even though the droplets (of diameter 20–40 μm) did not entirely occupy the channel of depth (100 μm), as shown in the inset of Fig. 2b, they formed a single layer. Since hexadecane droplets are lighter than the continuous aqueous phase (density difference Δρ = 230 kg/m³) they cream in the storage channel. The top wall of the channel confines this creamed emulsion and guides the droplets to form a 2D arrangement. Also, the dilution of the emulsion and flow of droplets during the injection from the Tygon tubing prevented the formation of multiple layers of emulsions and enabled the formation of a single layer of 2D emulsions in the storage serpentine channel.

The characteristics of the emulsions used in the cooling-heating cycles are listed in Table 1, where we also report their respective volume fraction, surface coverage in the storage channel and the flow conditions used to create the emulsion with the V-FFD. The approximate average diameters of these emulsions are noted as 20, 25, 32, and 40 μm throughout the text. Here, the volume fraction was determined by the ratio of the total volume of droplets in the field of view (blue rectangular box in Fig. 1b) to the corresponding volume of the storage channel. Likewise, the surface fraction was determined by the ratio of the circular area of all the droplet in the field of view to the corresponding area of the storage channel.

2.4. Emulsion temperature cycling experiments

The schematic of the experimental setup for emulsion cooling and heating experiments is shown in Fig. 1c. Briefly, the emulsion-loaded storage device was transferred to a Peltier based thermal stage (TSA12Gi, Instec) that mounts on the microscope [49]. The thermal stage has a 1.5 cm diameter circular window for optical access. A programmable controller (mk2000, Instec) and accompanying software (Wintemp, Instec) regulated the temperature of the device using a refrigerated water bath that acted as a heat sink [49]. To monitor the storage device temperature during the experiments, we placed two thin wire thermocouples (5SC-TT-K-40-36, Omega) in the temperature measurement ports located on the storage channel (orange lines in Fig. 1b). The temperature was recorded at 1-second intervals using a data acquisition board (TC08, Omega) and Omega Logging software 5.21.6.

The temperature protocol used for partial coalescence studies is shown in Fig. 1d. Initially, the thermal stage temperature was held at 23 °C. Then, the temperature controller was set to increase the temperature from 23 °C to 30 °C at the rate of 5 °C/min and held for 10 min to melt any potential crystallites. The temperature was then reduced to 2 °C at the rate of 1 °C/min and held there (for typically 20–90 min, depending on the droplet size) until all the liquid droplets were crystallized. Subsequently, the temperature was increased from 2 °C to 30 °C at the rate of 1 °C/min. During cooling-heating cycle, we acquired images of the droplet arrays at 24 fps at 10x magnification and resolution of 2 μm/pix.

The temperature was recorded at locations T1 and T2 for every experiment (Fig. 1b). In Fig. 1d, we show the temperatures recorded by the controller as well as the thermocouples T1 and T2. During the cooling step, there is a difference of 0.22 ± 0.03 °C between the controller temperature and the thermocouple measurements. The difference between the recordings of thermocouples T1 and T2 during the cooling-heating cycle was...
0.19 ± 0.02 °C. To determine the temperature variation within the storage channel we measured the temperature at three locations (Fig. S1a) in the serpentine channel (T1, T3, T4), in an empty device (i.e. with no emulsion). Results from these measurements are shown in Fig. S1b, where we see that the average variation between T1, T3, and T4 is 0.2 °C. However, in the middle of the device, T3 shows a variation of 0.6 °C during the coldest stage of the cycle. The larger temperature variation is consistent with the fact that the center of the storage channel is the farthest from the Peltier ring.

### 2.5. Image processing and data analysis

In both the droplet generation and the temperature cycling experiments, the droplet sizes and counts were measured using a custom-written image processing routine in MATLAB (R2016b, Mathworks). Fig. 2 shows the experimental image and the processed image of the droplets produced using the V-FFD (Fig. 2a) and the droplet array in the storage device (Fig. 2b). From the droplet size and count measurements in the V-FFD, we calculated the mean droplet size and polydispersity index (calculated as the coefficient of variance) of the emulsion for given operating conditions. From the droplet size and count measurements in the storage devices, we calculated the mean droplet size, polydispersity index, and the surface and volume fraction of the emulsions (see Table 1). We also calculated the percentage destabilization of the emulsion due to partial coalescence and the number of coalescence events due to a specified number of individual droplets merging with each other (discussed further below).

To detect the droplets in the images [50], we used the dark interfacial boundary resulting from the mismatch in the refractive index of the water and the oil phase. First, the circular boundary of droplets was identified by converting the images to binary. For more accurate detection of edges, the brightness and contrast of the images were adjusted. Depending on the sharpness of the circular boundary of droplets and the noise in the images, sharpening and softening functions were applied. Then, the images were magnified to ensure the circular liquid droplets were detected in each frame using the circular Hough transform [51]. The detected circles are shown in the bottom panels of Fig. 2a and b colored in blue. We verified the accuracy of the droplet detection algorithm by manually counting the droplets; the error in the number of detected droplets was less than 1%.

In the droplet generation experiments, when the droplet diameter exceeded the channel height, it assumed a pancake shape. Therefore, to convert the measured (or projected) circular diameters Dp to the actual droplet diameter D, we used the following equation [52]:

$$D = \left[ h^3 + \frac{3h}{2} (D_p - h) \left( \frac{\pi h}{2} + D_p - h \right) \right]^{\frac{1}{3}}$$

To verify the accuracy in using Equation (1), we have compared the droplet diameters obtained from Equation (1), with the droplet diameters measured outside the droplet generation device where they are spherical. We find the variation in droplet size estimated from these two independent approaches is less than 5%. The mean droplet size, i.e. diameter, and polydispersity index was calculated from a minimum of ≈ 600 droplets. The error bars in the plots indicate the standard deviation from the mean from three experimental replicates. For droplets in the storage device, they all take a spherical shape since the height (=100 μm) of the storage channel exceeds the droplet diameter.

To quantify the extent of emulsion destabilization, we calculate the percentage of coalesced droplets. The number of droplets at the end of the temperature cycle (Nf) can be compared with the number of intact droplets at the beginning of the cycle (N0) to estimate the percentage of coalesced droplets. However, we do not use this N0 value because we observe that during the heating step, coalescence of the partial crystalline droplets causes adjacent droplets to move out of the field of view (see Supplementary Video 2), thereby not preserving the initial N0. To quantify the actual number of individual droplets responsible for the destabilized emulsion state at the end of heating step, Nv, we use mass conservation. We back-calculate Nv as $\sum_{j} n_j$, where $n_j = \frac{\rho_j v_j}{\rho}$. Here, $v_j$ is the volume of the jth droplet in the destabilized emulsion at the end of the heating step and $\rho$ is the initial droplet volume. Although the number of individual droplets associated with a coalesced drop

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average diameter (μm)</td>
<td>19.14 ± 0.52</td>
<td>25.25 ± 0.97</td>
<td>32.75 ± 0.78</td>
<td>39.82 ± 2</td>
</tr>
<tr>
<td>Water flow rate (μL/hr)</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>Oil flow rate (μL/hr)</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>Valve pressure (Psi)</td>
<td>22.5</td>
<td>16.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of droplets</td>
<td>1801 ± 111</td>
<td>1135 ± 51</td>
<td>648 ± 8</td>
<td>486 ± 20</td>
</tr>
<tr>
<td>PDI (%)</td>
<td>2.0 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>Volume fraction (%)</td>
<td>4.7 ± 0.1</td>
<td>8 ± 0.3</td>
<td>10.0 ± 0.5</td>
<td>13.2 ± 0.6</td>
</tr>
<tr>
<td>Surface fraction (%)</td>
<td>42.0 ± 2.4</td>
<td>47.1 ± 0.8</td>
<td>45.5 ± 1.6</td>
<td>49.6 ± 0.5</td>
</tr>
</tbody>
</table>

PDI refers to polydispersity index.

Table 1: Characteristics of emulsions used in cooling-heating cycles, produced by V-FFD.

Supplementary Video 2.


3. Results and discussion

3.1. Droplet generation regimes and size control using the V-FFD

In this study, we employ the V-FFD with the goal of producing HIW emulsions of controlled size for emulsion destabilization studies, particularly with a focus on smaller droplet sizes. We anticipate that the V-FFD, with its ability to vary the orifice width, can provide this flexibility. To identify the relevant operating conditions, we examine the influence of dispersed phase flow rate ($Q_o$), continuous phase flow rate ($Q_w$) and valve pressure ($P_v$). First, we mapped the regimes of dispersed phase behavior without valve actuation and characterized the droplet size distribution. Subsequently, we pursued studies under valve actuation to investigate the flexibility that the V-FFD provides in tuning the droplet size in HIW emulsions (see Supplementary Video 1).

(a) Without valve actuation

With the valve unactuated, i.e. $P_v = 0$, we observed that the flow focusing device generates droplets in two main flow regimes which have been previously reported in the literature as dripping and jetting [53]. The dripping regime is characterized by the breakup of the dispersed phase at the orifice (see Fig. 3a); whereas in the jetting regime, breakup occurs after the dispersed phase forms a jet, downstream of the orifice (Fig. 3b). We scanned the oil and water flow rates and found that the dripping regime occurs in a narrower flow rate range than the jetting regime (Fig. 3c). We observe that while the dripping regime occurred predominantly at low dispersed phase flow rates, $Q_o < 50 \mu L/hr$, jetting behavior occurred mainly over a wider range of flow conditions, especially for $Q_o > 50 \mu L/hr$, as shown in Fig. 3c.

We quantified the droplet size for all the explored flow conditions. For the water flow rates in the jetting regime, we found that decreasing the oil flow rate significantly reduced the droplet size from 110 to 40 $\mu m$ as shown in Fig. 3d. With a further decrease in the oil flow rate to $Q_o = 50-100 \mu L/hr$, the regime switched to dripping in which the droplet size was much less sensitive to the oil flow rate (Fig. 3c). For instance, in the jetting regime at $Q_w = 100 \mu L/hr$, decreasing $Q_o$ three-fold from 150 to 50 $\mu L/hr$ decreased the droplet size from 85 to 55 $\mu m$. In the dripping regime, however, the same three-fold change from 15 to 5 $\mu L/hr$ decreased the droplet size from only 40 to 32 $\mu m$.

Our characterization of emulsions produced without actuation of the membrane valves shows that smaller droplet sizes could not be obtained in the jetting regime but can be obtained only in the dripping regime. However, the droplet size in the dripping regime could not be varied over a wide range due to insensitivity to flow rate, consistent with observations from prior works [33,54]. Also, without valve actuation, we found the dripping regime was rather narrow. To address these limitations, we used the V-FFD that can potentially expand the range of droplet sizes available in the dripping regime (including droplets with sizes less than 32 $\mu m$) by reducing the flow-focusing orifice width via actuation of membrane valves.

(b) Droplet formation in the V-FFD with actuated valve

We investigate the flexibility afforded in accessing smaller droplet sizes by actuating the valve channels and operating in the dripping regime observed in Fig. 3c (The black bounding box highlights these conditions). Specifically, we ran the V-FFD experiments by choosing a higher water flow rate $Q_w = 250 \mu L/hr$ and varying the valve pressure at set oil flow rates of $Q_o = 10, 15, 25$ and $50 \mu L/hr$. In Fig. 4a,b, we show that fixing the flow rates but
changing the valve pressure $P_v$ from 16.1 to 22.5 psi indeed lead to
a notable reduction in the droplet size.

In Fig. 4c, we show how varying the valve pressure from $P_v = 0$ to
30 psi influenced the regimes of droplet production for our chosen
flow conditions. Overall, we observed a broad regime of

3.2. Mechanisms involved in 2D emulsion destabilization due to partial
coalescence

The V-FFD device enables the production of monodisperse HiW
emulsions of controlled size. We loaded them into the storage
device and investigated the destabilization of the 2D emulsion
due to partial coalescence. The images in Fig. 5a show the three
main states of the 40 $\mu$m emulsion at a surface fraction of $\approx$ 50%.
At $T = 23$ °C, the droplets are monodisperse and in the liquid phase
(Fig. 5a, left). When cooling the emulsion at 1 °C/min to 2 °C and
holding for 20 min, all the droplets become solidified (Fig. 5b,
middle). Heating the emulsion leads to partial coalescence, finally
turning into a liquid emulsion with polydispersity size distribution
(Fig. 5c, right). In this section, we discuss the key mechanisms
responsible for emulsion destabilization that we discerned from
our experiments using the 40 $\mu$m emulsion as a representative
case.

(a) Destabilization through the formation of small- and large-
scale structures

In general, we observe that destabilization in the 2D emulsion
proceeds through two steps: (i) During the heating, droplets
appear to gradually melt which promotes the formation of small-
scale partially coalesced structures. The formation of these
structures is evident from the images shown in Fig. 5b, where from
$T = 17.0$ to 17.3 °C, partially coalesced structures with 2, 3, 4, 5
and 7 droplets are formed. In the images, these structures have
been color-coded so that their progression can be easily tracked.
Upon further increase in temperature from $T = 17.6$ to 18.1 °C,
these structures coalesce more and melt completely to form larger
spherical droplets. (ii) Because of the high surface coverage of the
2D emulsion, we also observe that large-scale multi-body units
form by coalescence of two or more previously formed smaller structures. This propagation of coalescence in a concentrated emulsion is one of the key steps to promote further destabilization of the emulsion. An example of coalescence propagation is shown in Fig. 5c where a 7-droplet structure coalesces with four of its adjacent droplets and then merges with additional neighboring structures to form a large-scale aggregate. Interestingly, similar multi-body coalesced structures have been reported in Pickering emulsions in the absence of phase transitions [55].

Our results show that even though small-scale partial coalescence events can spontaneously occur, to induce significant emulsion destabilization (similar to phase inversion) it is necessary to have the propagation of coalescence that leads to large-scale aggregates. We note coalescence propagation has been recently reported by Bremond et al. in a 2D liquid emulsion flowing in a microchannel [56]. They showed that a pair of droplets coalesce while separating due to bulging of interfaces [57]. Invoking this separation-induced coalescence mechanism, they argued that the shape relaxation due to the merging of two liquid drops drives separation of neighboring droplets triggering propagation of coalescence. In our case as well, there is shape relaxation of the partially coalesced structure during melting, however, we do not observe obvious bulging of interfaces probably because of the semi-crystalline elastic network in the melting droplets. Thus, new mechanisms might be involved in dictating the extent and limits of coalescence propagation.

(b) Restructuring of multi-droplet aggregates during coalescence

An important phenomenon occurring during coalescence is the restructuring of multi-droplet aggregates to a more compact form during the melting step. Dahiya et al. [45] reported a meniscus-driven restructuring mechanism that controls the stability of shapes formed when a third partially crystalline droplet is added to an arrested droplet doublet. They have shown that the expansion of meniscus which connects the droplets will result in the minimization of the surface energy of the triplet via movement of the third droplet to a more compact packing. They have noted that formation of a triangular packing with angles $\sim 60^\circ$ between the droplets is the densest possible arrangements of spheres based on a hexagonal close packing. This densest packing will not be achieved if coalescence arrest is stabilized at larger angles.

![Fig. 5. Process of emulsion destabilization during temperature cycling](image-url)
Depending on the solid fraction and initial approach angle of the droplets. Interestingly, similar bond angles of 60° have been reported by Studart et al. [58] during arrested coalescence of particle-stabilized droplets.

Similar to what Dahiyi et al. observed, we see the formation of necks that connect the droplets and rearrange them to a more compact form. For example, in the inset of Fig. 5b we follow the necks that connect the droplets and rearrange them to a more particle-stabilized droplets. The compaction appears to increase the distance with neighboring droplets thereby reducing the likelihood of small-scale structures to participate in coalescence propagation.

(c) Aggregate growth during coalescence propagation

Although the restructuring mechanism appears to limit coalescence propagation, we observe instances where large-scale multibody structures form (Fig. 5c and Supplementary Video 2). We suggest that coalescence propagation and growth may involve directional requirements since the large-scale 7-droplet structure shown in Fig. 5c seems to connect with only some of its adjacent droplets preferably. To probe this further, we followed the connections of this propagating multi-droplet structure, as shown in Fig. 6. Since the formation of connections is not very clear in the initial stages of coalescence at this level of magnification and image quality, at each step care is taken to highlight only the connections that obviously have a meniscus, meaning that the corners of the connection line of the two touching droplets are filleted. At T = 16.5 °C, 6 of these connections are observed and highlighted in yellow.

In the subsequent step (T = 16.7 °C), four more connections are recognized between the initial droplet structure and its surrounding droplets (highlighted in red) indicating coalescence propagation. The direction of these new connections with respect to the target droplet structure are shown with unidirectional black arrows. Similarly, the new connections formed at T = 16.9 °C are shown along with their unidirectional arrows. In the next step (T = 17.1 °C) the number of connections significantly increases due to the formation of connections between the target structure and other multi-droplet structures.

At 17.3 °C, the growth of the propagating structure becomes very limited, and the formation of new connections takes place only within the current structure, mainly by the addition of crosslinking connections which form equilateral triangles. These crosslinking connections that are internal to the target structure are shown by bidirectional arrows. Also, we observe that some of the structures do not form cross-linking connections (labeled as blue) and therefore do not participate in the growth process. Thus, the main conclusion we draw from analyzing the aggregate growth process is that the direction of coalescence propagation depends on the availability and orientation of the droplet structures adjacent to a propagating structure. Also, the formation of crosslinking connections which form equilateral triangles is the key to the stability and compaction of large-scale structures and noticeably limits coalescence propagation.

To summarize, destabilization in the 2D emulsion proceeds via formation of both small-scale structures and large-scale droplet structures which form through coalescence propagation. The compaction of the droplet structures due to meniscus-driven restructuring reduces the likelihood of coalescence propagation. In contrast, specific geometric orientations of neighboring droplet/structures appear to promote aggregate growth and favor coalescence propagation. Still, the coalescence propagation might be ultimately limited unless internal cross-linking connections are formed.

3.3. Effect of droplet size on destabilization of 2D emulsions

Given that we have identified the key mechanisms in emulsion destabilization, we use the capability of the V-FFD to generate monodisperse emulsions of various droplet sizes and investigate the effect of droplet size on the stability of emulsions to partial coalescence. We chose emulsions with mean droplet sizes of 20, 25, 32 and 40 μm, all having a similar surface fraction of ≈42–50%. Additional characteristics of these emulsions are listed in Table 1. We performed heating-cooling cycles on the microfluidic emulsions and quantified the degree of emulsion destabilization by evaluating the droplet size distribution in the emulsion at the beginning and end of the cooling-heating cycle. From the droplet size distribution, we also determined how many n-body coalescence events might have occurred that give rise to the final droplet size distribution (see Section 2.5).

In our system, we observe that destabilization occurs through a combination of spontaneous partial coalescence events (that yield
small-scale structures) and coalescence propagation (that yields large-scale structures). Although the formation of multi-droplet structures, both small- and large-scale, is visible from our coalescence movies, it is difficult to track how many structures form over time. Therefore, we did an endpoint analysis by plotting the probability $P(n)$ of $n$-body coalescence events for the four microfluidic emulsions, as shown in Fig. 7a–d, where we observe two main trends. First, we find that the fraction of intact droplets, $P(1)$, in the final emulsions decreases from around 90% in 20 μm droplets to almost 65% in 40 μm droplets. Second, we observe that the probability of occurrence of larger $n$-body structures increases with increase in the droplet size. For instance, in the 20 μm-emulsion, we do not observe any structures with >10 droplets (see Supplementary Video 3), while in the 40 μm-emulsion structures with as high as 58 droplets can form although with rather low probability. Both these observations indicate that emulsions with larger droplets undergo more coalescence, which increases their coalescence percentage - a measure of emulsion destabilization (Fig. 7e). A two-fold change in droplet size yields about a five-fold increase in emulsion destabilization (Fig. 7e).

The influence of droplet size on emulsion destabilization can be understood by considering how droplet size impacts the initiation of partial coalescence and coalescence propagation. Smaller droplets might be inherently more resistant to initiation of partial coalescence since the combined stress due to disjoining pressure and Laplace pressure needs to be overcome (Filip et al. [59]). In addition to droplet size affecting the probability of initiating partial coalescence, our results show that smaller droplets do not form large-scale multi-body structures. As we discussed before, the formation of these multi-droplet structures requires restructuring into more compact forms during coalescence. With smaller droplets, we expect that the higher curvature of the meniscus connecting them and their inherently less deformability (or higher Laplace pressure) would impose a high energy cost for this restructuring. With regards to coalescence propagation, since smaller droplets have higher energy cost for restructuring, it is less likely that

![Supplementary Video 3.](image-url)
In summary, the droplet size changes the probabilities of initiating partial coalescence, formation of multi-droplet structures, as well as coalescence propagation. This result is significant as previous explanations of emulsion destabilization due to temperature cycling focused largely on single droplet behavior. For example, studies suggest that larger droplets have larger interfacial crystals that protrude more, or they are more ‘reactive’, thereby increasing the chance of coalescence and its propagation [10,38]. Our study reveals that multi-droplet phenomena including restructuring and cross-link formation need to be incorporated to achieve a complete understanding of emulsion destabilization due to partial coalescence.

3.4. Potential mechanisms initiating partial coalescence

In our study, the focus is on understanding the mechanisms responsible for the 2D emulsion destabilization and how droplet size influences this process. Underlying the destabilization process is the spontaneous coalescence between two quiescent partially crystalline droplets. This initiation of partial coalescence appears to be not well understood. Walstra and co-workers [36] have argued that crystals at the droplet interface penetrate a neighboring partially crystalline droplet bridging the two to form a non-spherical structure. Another study suggested that the extent of protrusion of the interfacial crystals determines the ability of the droplets to partially coalesce [38]. More recently, however, it has been shown that coalescence can happen between partially crystalline droplets with no obvious crystal protrusions [40,60]. In our HiW emulsions, we do not observe noticeable crystal protrusions emanating from the drop surface. However, we observe that the droplet surface has a roughness which could be due to crystal partitioning which was previously reported by Ergun et al. [61]. These rough high-curvature regions could locally bring the droplet interfaces close together and trigger partial coalescence.

Another factor that might possibly contribute to the initiation of spontaneous coalescence is the changes in the solubility of surfactant due to crossing the Krafft temperature during the temperature cycling. The Krafft temperature of bulk SDS solutions (critical micelle concentration of SDS: 8 mM [62]) varies from 15.04 to 15.84 °C [63] for surfactant concentration varying from 50 to 85 mM. For the 2 wt% SDS solution (≈69.4 mM) we used, the Krafft temperature is estimated to be in the range of 15–16 °C. In our study, we observe that during the cooling step, the droplets are intact (i.e. stable) and the crystallized hexadecane droplets melt during heating at around 17 °C inducing destabilization. Thus, the destabilization temperature is above the Krafft temperature of SDS, suggesting that the partial coalescence-induced destabilization is not affected by the crystallization/solubility of SDS in the continuous phase. However, it is possible that the solubility of surfactant at the droplet interface might change due to the temperature cycling and may contribute to partial coalescence.

4. Conclusions

In this work, we successfully overcame the two main shortcomings of classical studies of partial coalescence [10,36–39,41–44], i.e. polydisperse droplets and indirect measurements, by generating monodisperse and size-tunable hexadecane—water microfluidic emulsions, and developing an approach to create 2D arrays of droplets to directly visualize the in situ destabilization of these emulsions during thermal cycling. This is significant considering the difficulties associated with partial coalescence studies [36,37] which rendered the underlying mechanism of how a population of droplets undergo destabilization unexplained.

The use of a V-FFD device in which the orifice width can be flexibly controlled by varying the valve actuation pressure enabled us to generate monodisperse HiW emulsions of droplet size ranging from 40–15 μm in the dripping regime. This expanded range of sizes toward smaller droplets afforded by V-FFD for generation of monodisperse HiW emulsions is significant. As we mentioned earlier, unlike monodisperse water-in-oil emulsions that have been generated at various sizes ranging from 7–200 μm [32], microfluidic monodisperse O/W emulsions have been achieved only over very limited size ranges, for example, 36 and 51 μm in one study [35] and 60–72 μm [17] in another.

Visualizing the destabilization of 2D arrays of these emulsions with a surface fraction of 40–50% showed that destabilization proceeds through a combination of spontaneous coalescence events that yield small-scale structures followed by formation of large-scale structures by coalescence propagation. We observe that before final destabilization, these multi-droplet aggregates restructure to a compact form with 60° bond angles. This re-arrangement was previously reported as the most compact form through which coalescence may proceed in partially crystalline and particle-stabilized droplets [45,58]. Also, we find that coalescence propagation depends on the availability and orientation of droplets and is controlled by the formation of crosslinking connections that unite the segments of the propagating structure via 60° bond angles.

By monitoring emulsion destabilization at four droplet sizes (20–40 μm) we found that the coalescence percentage increases almost linearly with droplet size. Quantifying the probability of n-body coalescence events revealed that the fraction of intact droplets is higher for smaller droplet sizes while larger droplets form multi-droplet structures with more members. Therefore, we concluded that the difference in the stability between various droplet sizes arises predominantly from the formation of droplet structures for which coalescence requires restructuring into more compact forms. We expect that higher curvature of the meniscus connecting droplets and higher Laplace pressure of smaller droplets would impose a high energy cost for this restructuring. Therefore, smaller droplet sizes are less likely to form large-scale multi-droplet structures, and consequently, they are more stable. These multi-droplet mechanisms have not been previously reported and extend the classical view of partial coalescence which is based mostly on single-droplet behavior [10,36–39,64,65]. Finally, the simplicity and versatility of our approach open new opportunities to understand not only emulsion destabilization but also a broader range of processes such as nucleation and formation of nonspherical structures.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcis.2018.08.045.

References
