Multiple Emulsions

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Microfluidic Mass-Transfer Control for the Simple Formation of Complex Multiple Emulsions**

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Multiple emulsions have complex internal structure in which the droplets of the dispersed phase themselves contain even smaller dispersed droplets.[1] They have significant potential in many applications, including pharmaceutics,[2,3] foods,[4,5] cosmetics,[6,7] microspheres,[8] microcapsules,[9–11] and chemical separations.[12–16] Double emulsions are typically formed through a two-step bulk emulsification process.[17] Owing to hydrodynamic heterogeneity in large-scale equipment, it is difficult to prepare multiple emulsions with a narrow droplet size distribution and precise microstructure.

Microfluidic devices provide improved hydrodynamic definition and thus enhanced control of multiple emulsions.[18–20] A variety of microchip formats have been investigated, including T-junction[21] and flow-focusing[22] designs. For example, a water–oil–water (W/O/W) emulsion was formed in microfluidic devices[23] by forcing a conventional W/O emulsion into a microchannel array. Double emulsions have also been produced by using this two-step emulsification process with straight-through microchannels,[24] although this method cannot precisely control encapsulation of the inner droplet. Cascading T-junction and/or flow-focusing designs[25–27] has also produced double emulsions, with some control over the number and size of the inner droplets, although device fabrication is complicated by the need for precise and local control of surface contact angle. Three-dimensional microcapillaries, which relax the surface wetting constraints, have been used to prepare both W/O/W and O/W/O emulsions. Utada et al.[28] developed coaxial flow-focusing geometries consisting of cylindrical glass capillary tubes nested within a square glass tube to produce double emulsions, and Chu et al. presented another microcapillary technique.[29] Lin et al.[30,31] developed a new microfluidic device that is able to produce double emulsions by incorporating pneumatically controlled moving-wall structures. However, all of these microfluidic methods seek to improve emulsion precision at the expense of more complicated device design. Additionally, such devices lack robustness because of practical flow-control limits in confined flows dominated by viscous and interfacial factors. Despite the enormous research effort to date, the preparation of precise double emulsions remains a significant research challenge, even on microfluidic platforms. New methods not dependent on hydrodynamic or mechanical control are urgently needed.

Herein we report a novel and simple method for turning a single emulsion into a double emulsion in a confined microfluidic flow. We show that the method can be extended to form multiple emulsions using only the T-junction microfluidic device format commonly used to form single emulsions.[21,32] Our method (Figure 1) relies on mass-transfer control of emulsion formation instead of more complex hydrodynamic or mechanical control, and specifically on initial solubilization of water in oil using a cosolvent, formation of a single emulsion at a T junction, and then autocatalytic formation of a double emulsion from the single emulsion through cosolvent shifting into the continuous carrier phase.

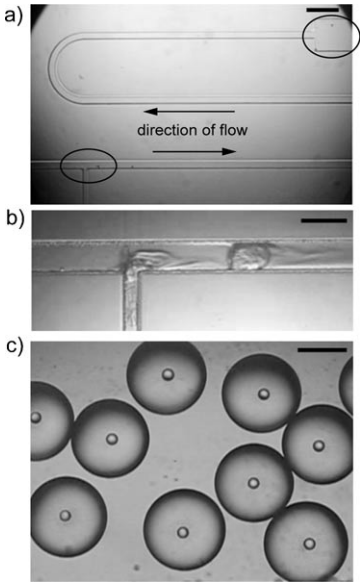

Figure 1. Multiple emulsions formed in a T-junction device. a) Optical micrograph of device geometry. Scale bar: 500 μm. Ovals enclose the T junction and expansion points. b) Droplet formation at the T junction. Scale bar: 100 μm. c) The resulting double emulsions at the flow expansion. Scale bar: 100 μm. Continuous phase: 100 μM peptide surfactant AFD4 with 200 μM $ZnSO_4$ pH 7.0; dispersed phase: ternary solvent Miglyol 812–ethanol–water (1:1:0.04 v/v/v). The flow rates of the oil and water phase were 0.01 and 1.0 mL h^{-1}, respectively.

Uniform W/O/W double emulsions were successfully produced with three fluids: Miglyol 812 oil, ethanol, and water. Miglyol 812, which is generally regarded as a pharmaceutically safe product,[33] is chosen as the dispersed oil phase, and water is used as the continuous phase. A ternary system

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comprising Miglyol 812 oil, ethanol, and water formed one homogeneous phase at a volume ratio of 1:1:0.04 v/v/v, and this system was used as the dispersed phase fluid. Peptide surfactant AFD4, which forms a cohesive, mechanically strong interfacial film[34] in the presence of zinc(II) ions at neutral pH, was added to the water continuous phase to prevent droplet coalescence. A typical process of formation of double emulsions in this T-junction geometry (Figure 1) is described as follows: The ternary dispersed-oil phase is first introduced from the perpendicular channel, whilst the continuous water phase flows through the parallel main channel. Once the dispersed phase penetrates into the main channel the droplets begin to grow, with ethanol in the oil phase shifting to the continuous water phase. The ternary oil droplet containing Miglyol 812 oil, ethanol, and water is then sheared off at the T junction, generating a droplet that has a blurred interface (Figure 1b), which is mainly due to rapid diffusion of ethanol from the oil phase to the continuous water phase. The time constant t_f for equilibration, that is, the time taken for ethanol in the droplet to transfer to the continuous phase, can be estimated by Equation (1):[35]

$$t_f \approx 50\, t_D\, Pe^{-2/3} \quad (1)$$

where t_D ($=h^2/D$, and h is the height of the channel, D is the diffusion constant) is the characteristic time of diffusion, and Pe is the Peclet number ($Pe=ud/D$, where u is the typical velocity, and d is the size of the droplet). For $D=10^{-9}\ m^2\,s^{-1}$, and droplet size $d=100\ \mu m$, Equation (1) predicts a diffusion time of 2.5 s at the flow rate of 1.0 mL h^{-1}. As it takes about 2 s for the droplet to travel to the expansion channel, and 200–500 ms for droplet formation, ethanol transfer from the oil to the continuous water phase will be nearly complete when droplets reach the downstream expansion channel, giving small water droplets inside the oil droplet (Figure 1c). Very regular W/O/W double emulsions with narrow size distributions for both the internal and external droplets were generated. The peptide surfactant succeeded in stabilizing the double emulsion against coalescence.[36] The external organic droplet size can be adjusted by the flow rate ratio of the dispersed to continuous phase, whilst the size of internal water droplets depends on the content of ethanol and water in the initial ternary oil phase.

This simple method also enables the formation of precise multiple emulsions. Using the same ternary Miglyol 812–ethanol–water oil phase with a continuous phase of water, we added two small non-peptide surfactants to stabilize the emulsions; one for the oil phase and another for the water phase. We chose the common surfactant sodium dodecyl sulphate (SDS) for the water phase at a concentration of 1 mM, instead of using the peptide surfactants designed in our lab,[34,37] and Span 80 (sorbitan monooleate, S6760, Sigma) for the ternary Miglyol 812–ethanol–water oil phase. O/ethanol–W/O/W triple emulsions were generated (Figure 2) with only a single emulsification step. In contrast to the double emulsion formation described above, the droplet showed a very clear droplet interface at the point of formation near the T junction (Figure 2a), which indicates that the ethanol in the oil phase did not diffuse as quickly as for the double emulsion

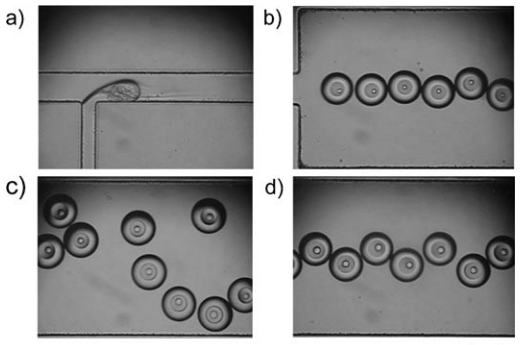

Figure 2. Formation of complex multiple emulsions with a T-junction device. a) Droplet formation at the T junction. b–d) Multiple emulsions formed using b) 14 mM, c) 16 mM, and d) 18 mM Span 80 (sorbitan monooleate, S6760, Sigma) in the dispersed phase. All the emulsions were made in the same device. Continuous phase: 1.0 mM SDS aqueous solution; dispersed phase: ternary Miglyol 812–ethanol–water (1:1:0.04 v/v/v) containing Span 80. The flow rates of oil and water phase were 0.01 and 1.0 mL h^{-1}, respectively. Scale bar: 100 μm.

lacking surfactant in the oil phase. The morphologies described herein are prepared reproducibly, and a series of monodisperse triple emulsions were prepared with different Span 80 concentrations (Figure 2b–d). The ability to produce triple emulsions with three different fluids within could allow novel materials to be produced without necessitating complex device redesign.

We further developed the method to form double emulsions with multiple fine internal droplets by increasing the surfactant concentration and changing the oil phase. A ternary sunflower oil, ethanol, and water mixture was used as the dispersed phase; sunflower oil is commonly used in food and cosmetic formulations. A 1 mM SDS solution was used as the continuous phase. It is however impossible to generate a homogenous dispersed phase owing to the low solubility of ethanol in sunflower oil. Therefore, sunflower oil was mixed with ethanol and water vigorously at a volume ratio 1:2:0.04 v/v/v in the presence of 20 mM Span 80, and it was ensured that partition equilibrium had been attained. The heavy phase was then collected, which contained a high concentration of sunflower oil with a certain amount of ethanol and water, as the dispersed phase. The double emulsion formed using our T-junction device (Figure 3) has many small droplets inside

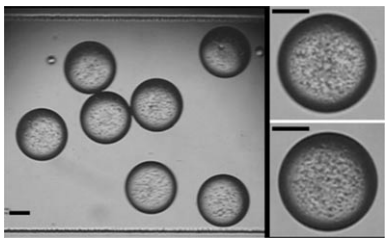

Figure 3. Double emulsions having complex internal structure formed using sunflower oil in a T-junction device. Continuous phase: 1.0 mM SDS aqueous solution; dispersed phase: the heavy phase of the ternary system sunflower oil–ethanol–water (1:2:0.04 v/v/v) formed with 20 mM Span 80. Flow rates of the oil and water phases were 0.01 and 1.0 mL h^{-1}, respectively. Scale bars: 50 μm.

owing to the high concentration of Span 80 in the oil phase. As Span 80 is not soluble in ethanol and water, it will partition primarily into the heavy phase, resulting in double emulsions with many fine inner droplets. Without any surfactant in the dispersed phase, double emulsions with one single inner droplet are formed (Figure 1), whereas for very high concentrations of surfactants in the dispersed phase, double emulsions are generated (Figure 3). In some cases, with an intermediate concentration of surfactant, monodisperse multiple emulsions are produced (Figure 2).

One of the major benefits of a double emulsion is the ability to encapsulate active ingredients that can be released from the inner phase to the outer phase by a controlled and sustained mechanism.[38] To demonstrate encapsulation, we loaded water-soluble methylene blue into a double emulsion, and observed a blue inner droplet inside a double emulsion (Figure 4a), whereas a white core was observed in the double

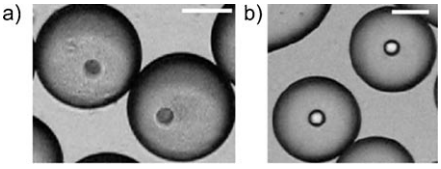

Figure 4. a) Microscope images of double emulsions encapsulating methylene blue, and b) double emulsions without methylene blue. Continuous phase: 100 μM AFD4 with 200 μM $ZnSO_4$ at pH 7.0; dispersed phase: the ternary system Miglyol 812–ethanol–water (1:1:0.04 v/v/v) with (a) or without (b) the presence of 0.072% w/v methylene blue. The flow rates of the oil and water phase were 0.01 and 1.0 $mL\,h^{-1}$, respectively. Scale bars: 50 μm.

emulsion lacking methylene blue (Figure 4b). When the oil droplets are generated, ethanol diffuses from the droplet to the continuous water phase, and Miglyol 812 adds an additional barrier that separates the innermost fluid from the continuous phase, preventing methylene blue and water from escaping the oil droplet. At 2 s, a single larger water droplet containing methylene blue is formed in the centre of the oil droplet, demonstrating that the water-soluble cargo remains encapsulated within the oil droplets. This result opens a simple method for the encapsulation of active chemicals.

In conclusion, we have shown that a T-junction microfluidic device can generate double emulsions having either a single internal droplet or many fine droplets. Furthermore, this simple and versatile approach also allows formation of multiple emulsions without the need for complex and difficult-to-manufacture microfluidic device geometries. This particular method has the ability to encapsulate substances in the inner water droplets, which is of great importance for potential delivery applications. Further work is needed to explore more solvent systems for the dispersed phase, because the appropriate selection of the dispersed phase chemistry is one of the most important steps in applying this new method.

Experimental Section

Peptide surfactant AFD4 (MW 2435) designed in our lab was synthesized by GenScript Corporation (Piscataway, NJ). The purity of AFD4 was > 95% by RP-HPLC. Peptide content was determined by quantitative amino acid analysis (Australian Proteome Analysis Facility, Sydney). Microfluidic chips were manufactured by Epigem Ltd. (Redcar, UK) from poly(methyl methacrylate) with a proprietary coating of aromatic epoxy. The dispersed phase was introduced from the perpendicular channel (width 50 μm), and the continuous phase was pumped from the parallel channel of width 100 μm. After droplets were sheared off at the T junction, they traveled downstream to a 500 μm expansion channel. Flow channels were 100 μm deep. Flow rates of continuous phase and dispersed phase were controlled using motor-driven syringe pumps (Harvard Pump 11 Plus, Harvard Apparatus, Holliston, Massachusetts). SGE glass syringes (Analytical Science Pty Ltd, Ringwood, Australia) were used for the continuous and the dispersed phase flow, respectively. Droplet pictures were recorded by video camera (Powershot A640, Canon Inc., Tokyo, Japan) mounted on an optical microscope (Eclipse 50i, Nikon Corporation, Tokyo, Japan).

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