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Stable Ultrathin-Shell Double Emulsions for Controlled Release

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Double emulsions are normally considered as metastable systems and this limit in stability restricts their applications. To enhance their stability, the outer shell can be converted into a mechanically strong layer, for example, a polymeric layer, thus allowing improved performance. This conversion can be problematic for food and drug applications, as a toxic solvent is needed to dissolve the polymer in the middle phase and a high temperature is required to remove the solvent. This process can also be highly complex, for example, involving UV initiation of polymeric monomer crosslinking. In this study, we report the formation of biocompatible, water-in-oil-in-water (W/O/W) double emulsions with an ultrathin layer of fish oil. We demonstrate their application for the encapsulation and controlled release of small hydrophilic molecules. Without a trigger, the double emulsions remained stable for months, and the release of small molecules was extremely slow. In contrast, rapid release was achieved by osmolarity shock, leading to complete release within 2 h. This work demonstrates the significant potential of double emulsions, and provides new insights into their stability and practical applications.

Double emulsions have long been considered as good candidates for the encapsulation and controlled release of various active ingredients, because of their compartmentalized core–shell structure.[1] The outer layer can act as a barrier to protect unstable active agents and control their release, and the inner core allows the encapsulation of active agents with a high loading capacity. Additionally, the outer liquid layer can also provide a compartment for encapsulating active agents that are poorly soluble in the inner core. However, the precise control over this compartmentalized core–shell structure remained a big challenge, until the advent of microfluidic technology.[2]

Monodisperse double or multiple emulsions are usually produced in two different ways in microfluidic devices. One way is similar to the traditional method for making double emulsions; W/O single emulsions produced in the first step are sequentially encapsulated in another aqueous phase, resulting in the formation of W/O/W double emulsions. This approach requires the precise modification of the local surface of the microchannel being formed to have both hydrophobic and hydrophilic junctions, allowing single W/O emulsions to be generated in the hydrophobic junction, followed by enclosure within the continuous water phase at the downstream hydrophilic junction.[2b] An alternative approach is the one-step microcapillary method.[2c] The device consists of two tapered cylindrical capillaries inserted into a square glass tube at each end. A coaxial flow forms with the innermost fluid pumped through one capillary tube from one end and the middle fluid flowing through the gap between the square tube and the capillary tube; the outermost fluid enters from the gap from the opposite direction then shears off the coaxial flow and generates double emulsion droplets, which are collected in the other capillary tube at the other end.[2c] This specific design allows the formation of a double emulsion or even multiple emulsions in one step.

Double emulsions can be produced by using microfluidic devices with precise control over not only the inner and outer droplet size, but also the number of inner droplets. However, two main problems hinder the practical application of double emulsions. Limited stability of double emulsions remains one of the major challenges. To circumvent this problem, the middle liquid shell is converted into a solid to allow long-term stability. Basically, polymers are dissolved in the middle solvent phase followed by the removal of the solvent to solidify the shell,[3] or monomers are added followed by crosslinking upon exposure to UV light.[4] These processes normally involve the use of toxic solvents to dissolve the polymers, elevated temperature for removing the solvent, or UV exposure. These elements of the processes are problematic for applications in foods, cosmetics, and pharmaceuticals, where the use of biocompatible components and more benign conditions are preferred. Therefore, to enhance their practical application, double emulsions made of biocompatible components that are first-and-foremost stable are required; secondly they must retain the encapsulated molecule inside until its release is deliberately triggered.

Herein, we report the generation of biocompatible ultrathin-shell W/O/W double emulsions with edible oils as the middle phase and significantly enhanced stability over months. Furthermore, we demonstrate their application in the encapsulation and controlled release of small hydrophilic molecules. Figure 1a shows the microcapillary device[5] for forming W/O/W double emulsion droplets with an ultrathin oil layer. The innermost aqueous phase is introduced from inlet 1. Discrete aqueous droplets are formed at the exit of the stretched capillary (inlet 1) and travel with the flow of the middle oil phase, which

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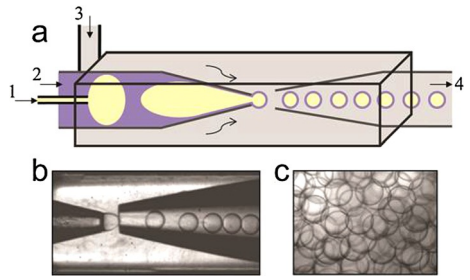

Figure 1. Formation of ultrathin-shell double emulsions. a) Schematic illustration of the microcapillary device for making ultrathin-shell double emulsions. b) Optical microscope image showing the formation of double emulsions. c) Optical microscope image of the double emulsions generated.

is injected from inlet 2. The inner surface of this left capillary (inlet 2) is treated to be hydrophobic, so a coaxial biphasic flow with the innermost aqueous droplets encapsulated in a thin layer of the middle oil phase is formed in the tapered cylindrical capillary at the left end (inlet 2); subsequently, W/O/W double emulsion droplets are generated at the exit as the coaxial biphasic flow is sheared off by the continuous aqueous phase (Figure 1 b). These are then collected in capillary outlet 4. Double emulsions with an ultrathin shell can be produced using various middle phases, such as hexadecane with 2% Span 80, poly(lactic acid) in toluene,[3a] a lipid mixture in 36 vol% chloroform, and 64 vol% hexane,[6] 8 wt% polystyrene in toluene.[3b] However, biocompatible oils have not yet been reported for making double emulsions; it still remains a challenge to use a viscous oil phase to make double emulsions.

To demonstrate the formation of biocompatible double emulsions, fish oil was used for the first time as the middle phase, and an aqueous poly(vinyl alcohol) (PVA; 10 wt%) solution was used as the outer- and inner-most phases. Fish oil is of great interest for food applications, owing to its health benefits.[7] This method can be easily adapted for other biocompatible oils, such as olive oil and sunflower oil. Previous studies have shown that the formation of double emulsions using viscous oil is nontrivial.[3a] We started with a flow-rate combination of 2000–200–200 μL h^{-1} (outer–middle–inner phase flow rates; F_o–F_m–F_i), and found that when the biphasic flow exited the left capillary, the thin layer of fish oil collapsed and no double emulsions were formed no matter what flow rates were used for the middle and inner phases (Figure 1 SA). When F_o was increased to 4000 μL per h, double emulsions were not generated under most of the conditions. In contrast, double emulsions could be produced at a F_o of 6000 μL per h or higher. The results indicated that higher flow rates benefited the formation of double emulsions. Generally, droplet formation depends on the dimensionless capillary number, $Ca = \mu U/\gamma$ (μ is the dynamic viscosity of the fluid, U is a characteristic velocity and γ is the surface or interfacial tension between the two fluid phases), which quantifies the competition between the viscous drag force and surface tension.[8] Although the behavior is more complex here, with three liquids, this simplified picture still provides valuable insight. The drag force pulls the droplet downstream, whereas the surface tension holds the droplet at the tip. When no surfactant is added to the middle oil phase, the interfacial tension is high. To break up the droplets, a large flow rate of the continuous phase is required to apply a strong drag force, which is consistent with the experimental observations. The size of the double emulsions can be tuned by F_o, but shows little dependence on F_m and F_i (Figure 1 SB). The size decreases, as the flow rate of the outer phase increases, in agreement with previous results.[3a]

The double emulsions were collected and observed by microscope (Figure 2). As both the outer and inner phases were 10 wt% aqueous PVA solution, there was no index mismatch and only the thin layer of the middle oil phase of each double emulsion appeared as a dark circle under optical microscopy. The double emulsions produced by using this method were uniform in size. Their stability was monitored visually; for capsules without any surfactant in the thin-shell fish oil, we found that double emulsions remained stable for only 8 h and all capsules disappeared after 24 h.

To improve the stability of these ultrathin-shell double emulsions, a surfactant was added to the middle oil phase. Initially, a commonly used, nonionic surfactant, Span 80, was added to fish oil. Unexpectedly, no double emulsions were formed and surprisingly no regular single emulsion formation occurred either. To explore this, the interfacial tensions of the four different systems were measured (Figure 3), including fish oil in water, fish oil in 10 wt% PVA, fish oil containing 2 wt% Span 80 in 10 wt% PVA, and fish oil with 1 wt% ABIL EM 90 in 10 wt% PVA. For fish oil in water without any surfactant, the interfacial tension started from 30 mN m^{-1} and dropped to an equilibrium value of around 20 mN m^{-1}, indicating the presence of surface-active components (such as fatty acids and so forth) in the fish oil. This observation explained the formation of double emulsions under a high flow rate of the continuous phase, as the interfacial tension was high and a high flow rate was required to initiate droplet breakup. The presence of PVA in the continuous phase decreased the equilibrium interfacial tension to 14.7 mN m^{-1}. However, we were surprised to find

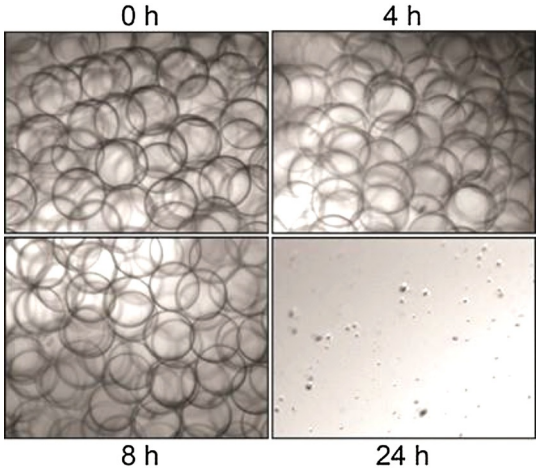

Figure 2. Stability of ultrathin-shell double emulsions of 10% PVA–fish oil–10% PVA that were generated at flow rates of 4000–400–800 μL per h. Without any surfactant, all the double emulsions broke up after 24 h.

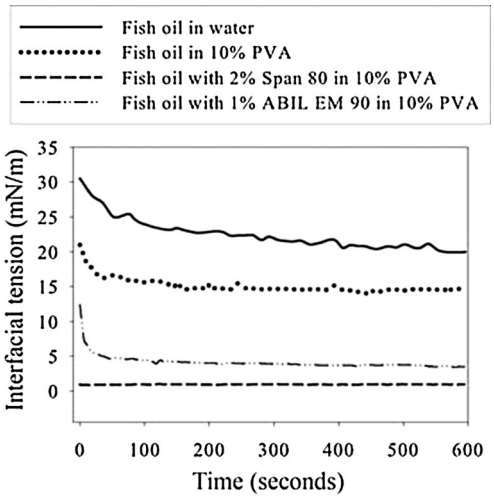

Figure 3. Interfacial tension of the fish oil with and without surfactant in 10 wt% PVA solution. The addition of Span 80 lowered the interfacial tension to 1.0 mN m^{-1}.

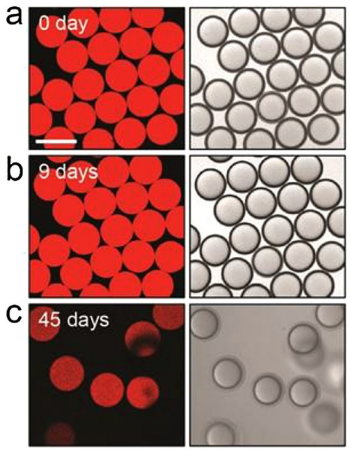

Figure 4. Long-term retention of a small hydrophilic molecule in the thin-shell double emulsions. Sulforhodamine B is used as a model molecule. Although the shell is thin, sulforhodamine B was retained in the double emulsions for long periods: a) 0 day, b) 9 days, and c) 45 days. The different fluorescent intensities of different double emulsions in (c) are due to their different depths. Scale bar: 250 μm.

the low interfacial tension (0.8 mN m^{-1}) of the system comprising fish oil containing 2 wt% Span 80 in 10 wt% PVA. Under such low interfacial tension, an unstable jetting regime forms under regular flow rates, leading to the formation of polydisperse droplets. To make uniform droplets in dripping mode, the flow rate needed to be reduced by about 15 times to match the reduction of interfacial tension;[9] such low flow rates of the continuous phase were not feasible for the microcapillary device used. Therefore, no double emulsions were observed. In contrast, the presence of another surfactant, ABIL EM 90, brought the interfacial tension down to about 5 mN m^{-1}, and monodisperse double emulsions of approximately 170 μm were produced at a flow rate combination of 4000–400–800 μL per h; this is much smaller than the double emulsion formed without any surfactant added (about 240 μm). With the addition of a small amount of surfactant in the oil phase, the stability of the double emulsions was greatly enhanced.

To demonstrate their application in encapsulation and controlled release, a small hydrophilic dye, sulforhodamine B, was dissolved in the innermost 10 wt% aqueous PVA phase and then encapsulated in the ultrathin-shell double emulsions. When emulsions were collected in a 100 mM sucrose solution, the dye was barely released as shown in Figure 4. After 9 days, the intensity of the dye remained almost the same when observed initially under confocal microscope (Figures 4a and b). The double emulsions were stable for months. The retention of sulforhodamine B inside the capsules was high. Even after 45 days, most of the sulforhodamine B remained inside the capsules and only around 20% of the dye was released, as indicated by the reduction of fluorescent intensity to about 80% of its original value.

Rapid release of sulforhodamine B from the W/O/W double emulsions was achieved by osmolarity shock (Figure 5). In this case, double emulsions were collected in water. At 0 min, the double emulsions showed an intense red color under confocal microscopy, indicating a high concentration of dye molecules in the core (Figure 5a). 15 min later, a significant amount of dye was released and the fluorescence intensity decreased by nearly half. Around 80% of the dye was released in the first 30 min (Figure 5b). At the same time, we found that the size of the double emulsion droplets increased, which indicates the inward flow of water, as a result of high osmolarity. The dye diffused out over time. After 120 min, almost all of the dye diffused to the outer phase and only approximately 10% of the dye was retained in the core of the double emulsion droplets. Therefore, under balanced osmotic pressure, as shown previously, the cargo can be encapsulated inside the thin-shell double emulsions for a long period of time and the release of the cargo can then be triggered by a change in osmotic pressure. This result shows that thin-shell double emulsions are ideal for applications that require stability and triggered release.

In this work, we report the fabrication of monodisperse double emulsions having an ultrathin layer of fish oil. This is the first report using edible oil as the middle phase to make stable double emulsions. Emulsion stability was significantly improved by using appropriate surfactants in the middle and continuous phase. As a model system, a small molecule was encapsulated in the aqueous core. Under well-controlled conditions, the molecules were kept inside the double emulsions for a long period of time, and even after 45 days a significant amount (approximately 80%) of the dye still remained inside the core. In contrast, cargo release was triggered by osmolarity shock, and nearly 50% of the small molecule was released in the first 15 min. This work demonstrates the feasibility of producing stable and biocompatible double emulsions and their applications in controlled release. The approach we used is likely not limited to a single type of oil or aqueous phase, allowing facile adaptation to other systems and potentially enabling diverse applications.

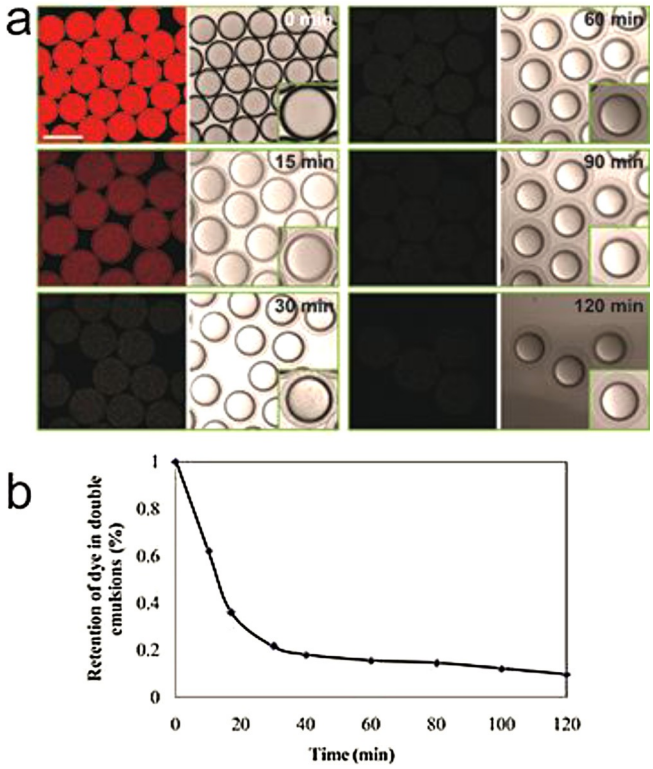

Figure 5. Triggered release of a model small hydrophilic molecule under osmotic shock. a) Confocal microscope images taken over time. b) Retention of the dye as a function of time.

Experimental Section

Double emulsions were produced in a glass microcapillary device,[3a,6] which was fabricated using two cylindrical capillaries (World Precision Instruments) of inner and outer diameters of 0.58 mm and 1.00 mm, respectively, and a square capillary (Atlantic International Technology, Inc.) with an inner width of 1.05 mm. The two cylindrical capillaries were tapered to a diameter of approximate 20 μm by using a micropipette puller (P-97, Sutter Instrument, Inc.), and then sanded to final diameters of 100 and 200 μm. Then the capillary with the diameter of 100 μm was treated with *n*-octadecyltrimethoxy silane (Aldrich) to make its surface hydrophobic, whereas the other capillary with a diameter of 200 μm was treated with 2-[methoxy(polyethyleneoxy)propyl] trimethoxy silane (Gelest Inc.) to render its surface hydrophilic. A third cylindrical capillary was stretched to an outer diameter of approximately 150 μm with a burner. Then the device was assembled on a microscope glass slide. Firstly, the square capillary was fixed to the slide with 5 Minute® Epoxy (Devcon). Then the two tapered capillaries were inserted in the opposite ends of the square capillary with the one having smaller diameter (100 μm) on the left and the one with larger diameter (200 μm) on the right. They were aligned under the microscope, separated by a distance of approximately 100 μm, and fixed to the side with epoxy. Next, the small stretched capillary was inserted into the left cylindrical capillary. Finally, dispersing needles, as inlets, were placed at the junctions and fixed to the slide with epoxy.

10 wt% aqueous solution of poly(vinyl alcohol) (PVA, MW 13000–23000) was used as the continuous and inner phases; fish oil (from Menhaden, Sigma) was used as the middle oil phase. 1 wt% ABIL EM 90 (Evonik Industries) was dissolved in the fish oil to reduce interfacial tension. A 100 mM aqueous solution of sucrose (VWR) was prepared to collect the emulsion droplets. The flow rates of the three phases were controlled by Harvard pumps (Harvard Apparatus). Thin-shell double emulsions were produced in a discontinuous dripping regime, which produced single emulsion droplets and ultrathin-shell double emulsions intermittently. To study the stability of double emulsions, 3 mL of the emulsions including single and double emulsions were collected in a 4 mL glass vial containing 1 mL solution of 100 mM sucrose. The single oil droplets and double emulsion drops were easily separated, due the difference in their densities, with the light oil droplets floating at the top, and the double emulsion drops suspended in the middle layer of the solution, as they have a similar density to the collecting solution. The double-emulsion formation in the microcapillary devices was recorded by using a high-speed camera (Phantom V7.3) mounted on top of an inverted microscope (Leica). Encapsulation of hydrophilic dye Sulforhodamine B in the inner droplets of the double emulsions was monitored using bright field and confocal fluorescence microscopy (Leica). All the experiments were performed at room temperature.

The interfacial tensions of fish oil with or without surfactant in water or 10 wt% PVA were measured using a Krüss Drop Shape Analysis System DSA10 (Krüss GmbH, Hamburg, Germany). An 8 mL quartz cuvette was filled with water or 10 wt% PVA, and fish oil droplets were formed from a U-shaped needle, as the oil phase is lighter than the aqueous phase. The dynamic interfacial data were collected by analyzing the drop shape with a reading taken every 2 s over 1000 s following the initial droplet formation.

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