

Monodisperse Water-in-Water-in-Oil Emulsion Droplets

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Since first being described by Seifriz in 1925,^[1] a double emulsion (also referred to as a multiple emulsion), water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) type double emulsions, have attracted significant research interest because of their potential applications in various fields, including agriculture, cosmetics, the food industry, extraction and drug delivery.^[2–12] In addition, double emulsions are often used as a template for the preparation of microcapsules with liquid cores.^[13–15] Microcapsules can be used to encapsulate drugs,^[16] living cells^[17] and are a promising technology for food,^[18] pharmaceutical^[16, 17, 19] and energy-saving^[20] applications.

Recently, efficient techniques for producing highly monodisperse W/O/W and O/W/O emulsions have been developed.^[21–24] Microfluidics techniques that utilize microchips, flow-focusing devices and coaxial microcapillary fluidic devices can enable precise control of the outer and internal drop sizes. Monodispersibility should make double emulsions highly desirable for the applications mentioned above. There are still unattained requirements for the production of double emulsions and for the composition or properties of the interior phase. In production, the requirement is to simplify the emulsification process. To fabricate monodisperse double emulsions using microfluidic devices, one must precisely and continuously control the flow rate of the innermost, middle, and outermost fluids because the size of the outer and inner droplets is fixed by the shear stress arising from each fluid flow. In general, different thicknesses of the internal phase occur in double-emulsion globules because it is difficult to maintain the internal phase at the center of the globules.

Herein, we report on a new class of double emulsion that can overcome the above disadvantages. The double emulsion is prepared via an aqueous two-phase system. Aqueous two-phase systems were originally observed in polymer solutions and are generally composed of a water solution of two particular polymers. At high concentrations of these components, spontaneous phase separation takes place. One phase is rich in one polymer and the second phase is rich in the other polymer. The phase separation phenomenon is interpreted in

terms of the incompatibility of polymers. When two entirely aqueous solutions containing different water-soluble molecules are mixed together, a water-in-water (W/W) emulsion is generated, which is a system that consists of a polymer suspended as water-solvated droplets dispersed in a solution of another polymer whose solvent is also water.^[25–28]

Two types of W/W emulsion are shown in Figure S1 (Supporting Information), made from aqueous solutions of polyethylene glycol (PEG) and Dextran (DEX) at different concentrations. To clearly demonstrate each phase, a water-soluble dye, reactive blue 180, which preferably partitions into the PEG-rich phase, was added to the system. In a W/W emulsion, the polymer solution having the larger volume fraction tends to become the continuous phase, within which the other polymer solution is dispersed as droplets. When the W/W emulsion is added to an organic solution and shear is induced, the W/W emulsion then disperses in the organic solution as droplets, forming a water-in-water-in-oil (W/W/O) emulsion. PEG is a synthetic, low-molecular-weight (MW), non-ionic, linear polyoxyethylene polymer. DEX is a high-MW, highly polar, linear polysaccharide polymer produced by certain species of bacteria. PEG is less polar than DEX, presumably because of its partial aliphatic hydrocarbon composition. Thus, in two-phase PEG/DEX systems, the PEG-rich phase is generally considered to be more hydrophobic than the DEX-rich phase.^[29] Therefore, when a W/W emulsion consisting of an aqueous PEG/DEX two-phase system is dispersed in an organic solution, the lower polar PEG-rich phase strongly attracts the organic solution through hydrophobic interactions. As such, the middle phase of a double emulsion results from the PEG-rich phase (Figure S1, Supporting Information).

This phenomenon relies on thermodynamic spreading equilibria between two fluids suspended as emulsified droplets in a solvent. Pioneering work by Torza and Mason established the tendency of a liquid to spread spontaneously across a solid or liquid surface in terms of the surface and interfacial tensions of the components and they could then represent such a system in which two dissimilar phases are dispersed within a third by substituting the appropriate interfacial tensions for the surface tensions [Eq. (1)]:^[30]

$$\lambda_{(\text{PEG}-\text{DEX})} = \gamma_{(\text{DEX}-\text{Org})} - \gamma_{(\text{PEG}-\text{Org})} - \gamma_{(\text{PEG}-\text{DEX})} \quad (1)$$

where $\gamma_{(\text{PEG}-\text{Org})}$ and $\gamma_{(\text{DEX}-\text{Org})}$ are the interfacial tensions between the organic solution and the PEG-rich phase and DEX-rich phase, respectively; and $\gamma_{(\text{PEG}-\text{DEX})}$ is the interfacial tension between the PEG-rich phase and the DEX-rich phase. For positive values of the spreading coefficient $\lambda_{(\text{PEG}-\text{DEX})}$, the PEG-rich phase will spontaneously spread on the organic phase. The

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Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/cphc.201000905>.

configuration of the two polymer phases (PEG and DEX) will depend on the values of the three possible spreading coefficients and can be described as complete engulfing, partial engulfing and non-engulfing.

Combining the emulsion theory described above with the phenomenon of phase separation provides a novel approach for preparing W/W/O double emulsions. By introducing a two-polymer solution into a continuous organic phase, a stable emulsion is created in which phase separation occurs within each droplet. The phase separation is engineered such that one polymer-rich phase spreads and completely covers the O/W interface, controlling the interior of the double emulsion. If a homogeneously mixed W/W emulsion is used, the volume fraction of the middle phase in the inner core of the resultant W/W/O emulsion should become constant for all droplets fabricated in the same batch. If the well-mixed W/W emulsion is compartmentalized and uniform in size, the volume and the thickness of the middle phase, as well as the volume and the size of inner core in the resultant W/W/O emulsion, must become uniform. Moreover, the concentrations of each polymer in both aqueous phases can be thermodynamically determined and predicted from the phase diagrams of the aqueous two-phase systems.^[31–33] This thermodynamic partition equilibrium allows us precise control of the interior of the W/W/O emulsion droplet and thus we can achieve a high degree of control over the resultant double emulsions. Homogeneously mixed W/W emulsions consisting of different compositions of PEG and DEX were introduced into a flow of hexadecane at a Y-shaped junction in a microchannel, which was used to fabricate monodisperse aqueous two-phase droplets. We successfully produced uniform W/W/O emulsion droplets, in which each drop contained a single internal droplet, creating core-shell structures in which the internal and external drop diameters and the thickness of the middle phase were highly controlled (Figure 1). Microfluidic emulsification offered monodisperse W/W/O emulsion droplets of diameter 96 μm (CV = 4.4%), with monodisperse inner aqueous droplets with a diam-

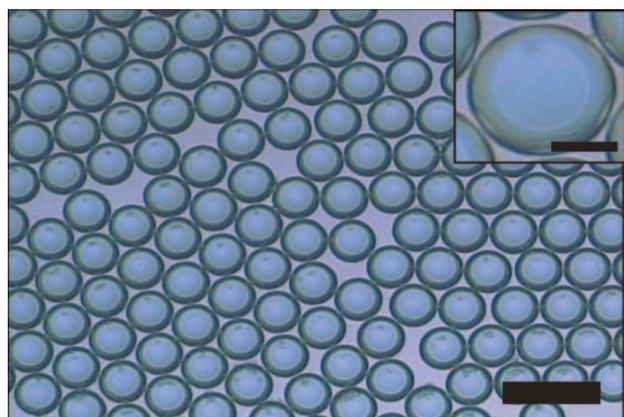


Figure 1. Monodisperse W/W/O emulsion droplets prepared by emulsification of a W/W emulsion in a Y-shaped microchannel. W(inner): DEX-rich phase, W(middle): PEG-rich phase, O: Hexadecane containing 3 wt% Span80. The concentration of PEG/DEX was 7.7/9.7 (w/w). The scale bar is 200 μm . The inset is a magnified view with a scale bar corresponding to 50 μm .

eter of 67 μm (CV = 6.6%). Alternatively, by changing only the fraction of PEG-rich phase to that of the DEX-rich phase, we could control the size of the internal core, that is, control the thickness of the middle phase (Figure 2). It is worth noting

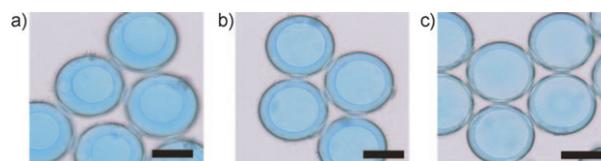


Figure 2. W/W/O emulsion prepared by emulsification of a W/W emulsion in hexadecane containing 3 wt% Span80 in a Y-shaped microchannel, used to fabricate monodisperse emulsion droplets. The W/W emulsion was prepared by using different volumes of aqueous solutions of 12.5 wt% PEG and 25.5 wt% DEX a) 9.0/7.7, b) 7.7/9.7 and c) 6.6/12.1 (w/w). Scale bars are 50 μm .

that, irrespective of the volume fraction of the PEG- and DEX-rich phases, the middle phase consisted of the PEG-rich phase: the PEG-rich phase became the middle phase even if it was the dispersed phase in the initial W/W emulsion. That is, the PEG-rich phase took the place of the DEX-rich phase, adsorbed onto the O/W interface, and expanded on the O/W interface.

Phase separation and generation of the middle and internal phases in the W/W/O emulsion droplet was continuously observed (Figure 3). When the volume fraction of the PEG-rich

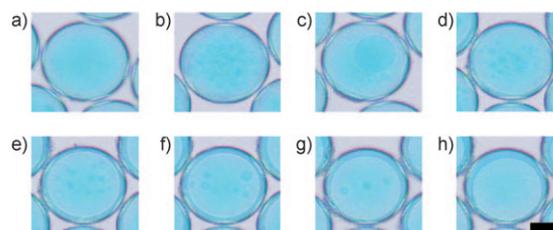


Figure 3. Time dependence for the development of W/W/O emulsion droplets: a) 60 s, b) 840 s, c) 960 s, d) 1140 s, e) 1260 s, f) 1380 s, g) 1560 s, h) 1860 s. The concentration ratio of PEG/DEX was 6.6/12.1 (w/w). The scale bar is 25 μm .

phase was smaller than that of DEX-rich phase, tiny PEG-rich droplets, which were homogeneously dispersed in the DEX-rich phase, aggregated and simultaneously adhered and expanded on the O/W interface. Movement of the PEG-rich droplet to the O/W interface seems to be a random diffusion governed by Brownian motion. However, Brownian motion is not the dominant source. The droplet is also influenced by long-range hydrophobic attractions from the external organic solution and should spontaneously diffuse to the O/W interface.^[34] The photographs shown in Figure 4 are a time course of the interior of W/W/O emulsion droplets fabricated with W/W emulsions consisting of large volume fractions of the PEG-rich phase. Contrary to the W/W/O emulsion shown in Figure 3, homogeneously dispersed, tiny DEX-rich droplets aggregated but did not adhere on the O/W interface. The DEX-rich droplet

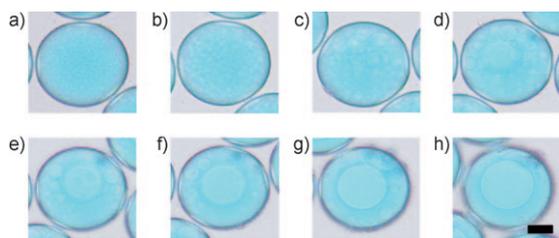


Figure 4. Time dependence for the development of W/W/O emulsion droplets: a) 60 s, b) 150 s, c) 240 s, d) 330 s, e) 420 s, f) 510 s, g) 600 s, h) 900 s. The concentration ratio of PEG/DEX was 9.0/7.0 (w/w). The scale bar is 25 μm .

spontaneously moved to and aggregated at the center of the W/W/O emulsion droplet. The resultant DEX core was totally isolated from the O/W interface because there was a PEG-rich liquid membrane immobilized around the O/W interface by hydrophobic attraction. As mentioned above, the nature of the W/W/O emulsion is fundamentally different from common W/O/W and O/W/O type emulsions. Because the hydrophilic/lipophilic nature between the external phase (continuous phase) and the internal cores of W/O/W and O/W/O emulsions are similar, the internal core has a tendency to be attracted toward the O/W interface between the external phase and the middle phase. Furthermore, the specific gravity of the internal core is quite different from that of the middle phase. For such reasons, the internal core cannot keep its position at the center of the double emulsion droplet. On the other hand, the position of the internal core in a W/W/O emulsion is nearly at the center of the droplet. As stated above, the internal core of the W/W/O emulsion droplet is not attracted toward the external phase because of the middle barrier phase. In addition, the specific gravity of the internal core is almost same as that of the middle phase. The small forces of gravity and buoyancy are not strong enough to allow the internal core to displace the middle phase immobilized by the hydrophobic attraction. As a result, the internal core is thermodynamically stable at the center of droplet. The central location of the internal core in the double emulsion droplet means that the thickness of the middle phase is uniform everywhere in the droplet. Regardless of whether the double emulsion is used as is or after encapsulation, uniform thickness of the middle phase is an essential requirement for controlled release of chemicals from the internal core. Furthermore, the W/W/O emulsion is expected to be a promising tool for the fabrication of microcapsules. Microcapsules fabricated from W/W/O emulsions should possess other advantages over those of W/O/W and O/W/O emulsions besides the central location of the core. The ability to transfer hydrophilic ingredients through the middle phase is different in microcapsules originating from W/W/O emulsions compared with those that originated in W/O/W emulsions because of the barrier caused by the middle phase of the emulsion. For microcapsules fabricated from W/O/W emulsions, the permeability of hydrophilic ingredients is limited by the water-repellent matrix of the shell, which is synthesized from a lipophilic monomer. In the case of microcapsules fabricated from W/W/O emulsions, the shell is easily swollen by water and therefore

the permeability of the ingredients, which is determined by the density of the shell (concentration of polymer), is facilitated. In addition, because the aqueous two-phase system was developed during the mid-1950s for the separation of macromolecules, cells and organelles,^[35,36] there are a number of experimental observations^[37–39] and theoretical models^[40,41] that describe the bulk phase composition and solute partitioning behavior of cells, particles and proteins in aqueous PEG/DEX two-phase systems. Such highly selective partitioning ability should allow effective encapsulation of solutes in the microcapsule. These properties are expected to be advantageous in food and pharmaceutical applications.

Experimental Section

Water-in-Water Emulsion Composed of the Polyethylene Glycol/Dextran Aqueous Two-Phase System: Different volumes of aqueous solutions of 30 wt% polyethylene glycol (PEG, Mw 4000 g mol^{-1}) and 30 wt% dextran (DEX, Mw 32 000–45 000 g mol^{-1}) were mixed by vortexing or homogenizer. The volumes of the aqueous solutions were determined to give the desired amounts of PEG and DEX in the emulsion. To clearly demonstrate each phase, a water-soluble dye, reactive blue 180, which preferably partitions into the PEG-rich phase, was added to the aqueous two-phase system. **Monodisperse Water-in-Water-in-Oil Emulsion:** All experiments we report here used a grass device after hydrophobic surface treatment by 1-octadecyltrimethoxysilane. Water-in-water emulsions were prepared by using different volumes of aqueous solutions of 12.5 wt% polyethylene glycol and 25.5 wt% dextran, then added to an organic phase. The organic phase was hexadecane, containing 3 wt% Span80. Droplet formation was performed by using a Y-shaped microchannel. Microfluidic emulsification offered precise control of highly monodispersed emulsion droplets (Figures 1, 2). An illustration of the channel is shown in the Supporting Information. The water-in-water emulsion and the organic phase were each introduced into the microchannel through inlets 1 and 2, respectively, using a syringe pump. A teflon tube (ϕ 500 $\mu\text{m} \times 11 \text{ cm}$) was connected at the outlet to collect W/W/O emulsion droplets.

Keywords: emulsions • free-energy relationships • liquids • polymers • polysaccharides

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Received: October 28, 2010

Published online on January 18, 2011