21
Double, Triple and Complex Multilayered Emulsions

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21.1
Introduction

Emulsions with complex multilayered structures are referred to as multiple emulsions. A well-known example is a double emulsion, in which microdroplets that enclose even smaller droplets are suspended in a continuous liquid phase [1–3]. Figure 21.1 shows the two main types of double emulsions: water-in-oil-in-water (W/O/W) emulsions, in which a water-in-oil (W/O) emulsion is dispersed in an aqueous phase (Figure 21.1a) and oil-in-water-in-oil (O/W/O) emulsions, in which an oil-in-water (O/W) emulsion is dispersed in an oil phase (Figure 21.1b). Usually, both hydrophilic and lipophilic surfactants are required to stabilize these multilayered dispersions.

Both W/O/W and O/W/O emulsions have attracted considerable attention because of their potential applications in food science [4–7], cosmetics [8–10] and pharmaceutics [11]. In particular, there have been many studies on the pharmaceutical applications of W/O/W emulsions because the internal aqueous droplets can contain water-soluble drugs for controlled release or targetable delivery [12–14]. Solid microcapsules loaded with bioactive polymers are also prepared from W/O/W droplets by the solvent evaporation method [15–18]. Other applications studied thus far include the synthesis of shaped polymeric microparticles [19] and the use of the intermediating phase as the permeation membrane in separation technology [20–25].

Double emulsions are conventionally manufactured through two-step bulk emulsification [26] (Figure 21.2). In the first step, a primary single emulsion (e.g. W/O or O/W emulsion) is produced through the high-shear mixing of two immiscible liquids (Figure 21.2a). Then, the single emulsion is gently emulsified within a third immiscible liquid under low-shear conditions in order to prevent the disruption of the initial single emulsion and the produced double emulsion droplets (Figure 21.2b). This technique is widely used in industry because it is simple and easy to use. In this technique, however, it is difficult to produce monodisperse double emulsions due to the lack of control over the droplet size and droplet size distribution

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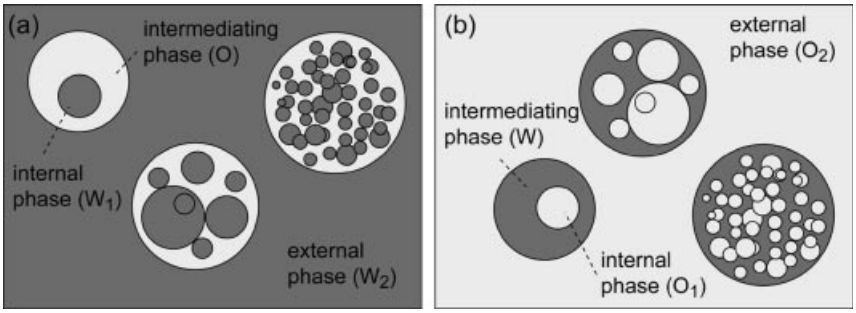

Figure 21.1 Schematic illustration of typical double emulsions. (a) $W_1/O/W_2$ emulsion: (b) $O_1/W/O_2$ emulsion.

in both the emulsification steps. Phase inversion [27–31] and other conventional techniques [32, 33] also have similar drawbacks. On the other hand, several new methods that utilize various microfabricated structures have recently been developed for producing quasi-monodisperse or highly monodisperse double emulsions. Moreover, some of these methods can precisely control the size, structure and composition of the double emulsions.

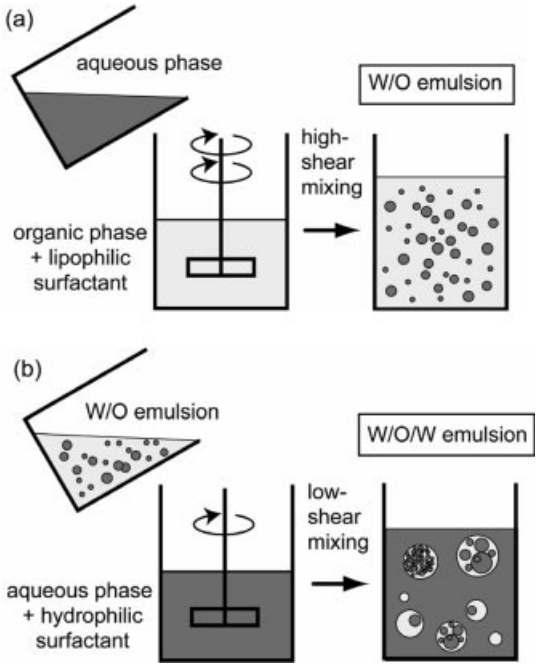

Figure 21.2 Schematic illustration of the two-step homogenization for producing W/O/W emulsions. (a) Step 1: high-shear mixing to produce a fine W/O emulsion. (b) Step 2: low-shear mixing to produce a W/O/W emulsion.

In this chapter, we describe the new technologies that can produce various double emulsions of controlled sizes, structures and compositions. We also explain the emerging new applications of the monodisperse double emulsions prepared using those technologies. Section 21.2 describes the use of porous materials for emulsification membranes. Section 21.3 describes the use of a channel array or through-holes fabricated on a silicon substrate. Section 21.4 describes the use of microfluidic channels on a planar substrate. Section 21.5 explains coaxial microcapillary devices. Section 21.6 describes the applications of monodisperse multiple emulsions to a new class of functional materials.

21.2 Membrane Emulsification

Membrane emulsification [34–39] is a technique developed in the 1980s that employs porous membranes to form emulsions. Among the several porous materials available, Shirasu porous glass (SPG) is a well-known permeation membrane because it has pores with narrow size distributions and the pore size can be precisely controlled in the range from sub-micrometers to tens of micrometers. Nearly monodisperse emulsion droplets are formed by forcing a phase to be dispersed into a continuous phase through the pores on the membranes. The coefficient of variation (CV) of the droplet diameters, which is calculated as the standard deviation of the droplet diameters divided by the mean droplet diameter, is generally around 10%. The droplet size depends on the pore size and the linear relation is given by the following equation:

$$D_d = aD_p \tag{21.1}$$

where D_d is the average droplet diameter, D_p the average pore diameter and a the proportionality coefficient, which can range typically from 2 to 10 [35, 38]. The hydrophilic surface of SPG is suitable for the production of O/W emulsions because the organic droplets can easily detach from the membrane surface. For producing W/O emulsions, the SPG surface is chemically modified to be hydrophobic by a silane coupling agent [e.g. octadecyltrichlorosilane (OTS)]. The SPG membrane emulsification is applied commercially to various products [35], such as low-fat spreads, polydivinylbenzene microspheres [40] for spacers in liquid crystal displays and silica powder for liquid chromatography. Various SPG emulsification devices are now commercially available [41].

Nearly monodisperse double emulsions can be prepared using membrane emulsification by using a single emulsion as the phase to be dispersed [42, 43]. The primary single emulsion is usually prepared by conventional homogenization, although the use of two-step membrane emulsification (Figure 21.3) has also been proposed [35]. The preparation of W/O/W emulsions by using membrane emulsification was first reported by Mine *et al.* [42]. First, they used conventional homogenization to prepare a W/O emulsion with a mean diameter of 0.54 μm. This W/O emulsion was then forced into the external aqueous phase through a microporous

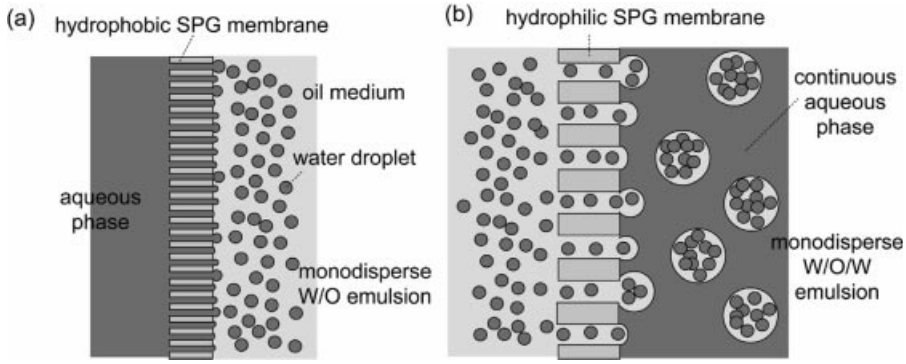

Figure 21.3 Preparation of a monodisperse W/O/W emulsion by using two-step membrane emulsification. (a) Preparation of the W/O emulsion by using a hydrophobic membrane with smaller pores; (b) preparation of the W/O/W emulsion by using a hydrophilic membrane with larger pores.

glass membrane with a mean pore size of 1.0 μm, producing W/O/W emulsion droplets with a mean size of 4.8 μm. The W/O/W emulsions thus prepared were stable for at least 6 weeks when stored at low temperature (298 K).

Higashi and coworkers [44–47] used SPG membrane emulsification to prepare W/O/W emulsions for arterial injection chemotherapy of liver cancer. By means of sonication, they produced a sub-micron W/O emulsion in which aqueous droplets containing epirubicin, a water-soluble anticancer drug, are dispersed in iodized poppy seed oil (IPSO), which selectively deposits itself on the cancerous tumor. They then used the SPG membrane to produce quasi-monodisperse IPSO droplets with diameters of 30.1 ± 5.1 μm (CV $\approx$ 16.9%) or 70.0 ± 6.7 μm (CV $\approx$ 9.6%) in order to encapsulate the aqueous phase containing the anticancer agent. Finally, they used the W/O/W emulsions for clinical applications and reported that the IPSO droplet size determines the anticancer effect.

Membrane emulsification is also used for producing a fine W/O/W emulsion from a coarse W/O/W emulsion that has been prepared using the conventional two-step homogenization [48–50].

The preparation of double emulsions by using membrane emulsification is comprehensively described in a review by van der Graaf *et al.* [51].

21.3 Microchannel (MC) Emulsification

Microchannel (MC) emulsification [52–58] is a technique developed in the 1990s, which utilizes lithographically fabricated geometries for producing monodisperse emulsion droplets. Thus far, two configurations have mainly been studied: one consists of a micron-sized comb-like channel array microfabricated on a silicon plate [52–54], whereas the other is called a straight-through MC plate and has thousands of

microfabricated through-holes with oblong cross-sections [55]. In both configurations, monodisperse droplets with CVs of diameters below 5% can be formed by forcing a phase to be dispersed into a continuous phase through the arrayed channels having the same sizes. In this technique, the formation of droplets is induced by interfacial tension [54] and the droplet sizes can be adequately controlled in the range from a few micrometers to ~100 μm, by changing the sizes of the microfabricated structures. The hydrophilic surface of the silicon substrates is suitable for producing O/W emulsions, whereas a hydrophobic surface is required for W/O emulsions.

The preparation of double emulsions by using MC emulsification was first reported by Kawakatsu *et al.* [56]. In the first step, a W/O emulsion, which was used as a feed emulsion, was prepared by conventional homogenization. It was then forced into the microchannel array on a silicon substrate to produce a W/O/W emulsion. Solid-in-oil-in-water (S/O/W) pectin microcapsules were also formed by the gelation of the internal aqueous phase – the pectin solution – using a calcium solution containing Tween 20 as an external water phase.

Sugiura *et al.* [57] also produced nearly monodisperse W/O/W emulsions by using a two-step emulsification process employing MC emulsification as the second step (Figure 21.4). They tested four organic fluids (decane, ethyl oleate, medium-chain triglyceride and triolein) as the middle organic phase. W/O emulsions, which were used as feed emulsions, were prepared by conventional homogenization and had diameters of 17.9–21.0 μm with CVs of 26.0–29.6%. They then prepared W/O/W emulsion droplets having diameters of 31.8–35.7 μm with CVs of 5.5–19.0% by using MC emulsification. The mild emulsification in this technique led to a high entrapment yield of the W/O/W emulsion, which was determined fluorimetrically as 91%.

Kobayashi *et al.* [58] prepared monodisperse W/O/W emulsions having internal aqueous phase contents of 10–30% by using a two-step emulsification using straight-through MC emulsification as the second step (Figure 21.5). A homogenizer and a microfluidizer were used to produce sub-micron W/O emulsions having diameters

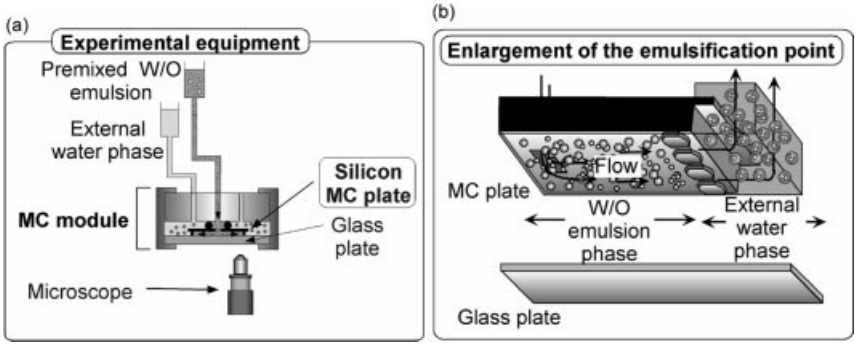

Figure 21.4 Schematic of the MC array device for producing a W/O/W emulsion. (a) Equipment; (b) formation of W/O/W droplets at a microfabricated array. Reprinted from Ref. [57], Copyright 2004, with permission from Elsevier.

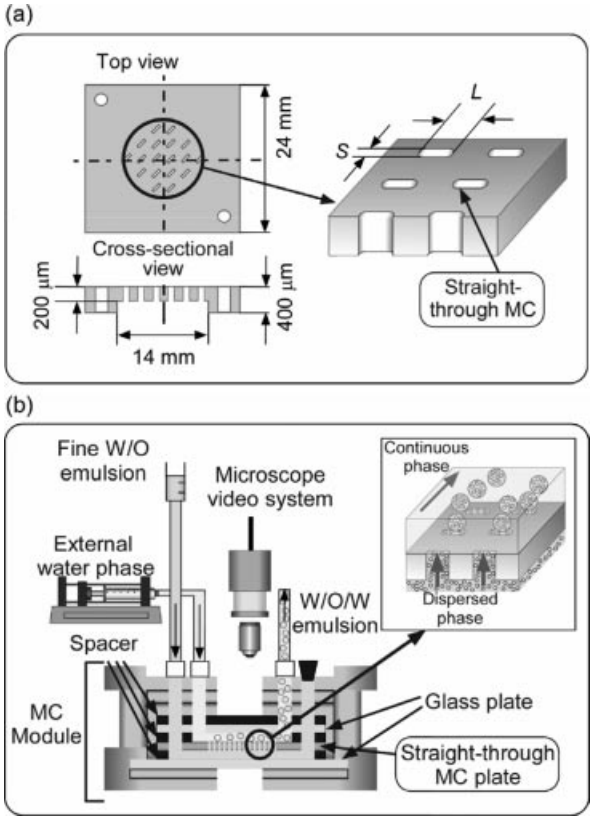

Figure 21.5 Schematic of the straight-through MC emulsification device used for producing monodisperse double emulsions. (a) A straight-through MC plate; (b) an experimental setup for producing W/O/W emulsions. Reprinted from Ref. [58], Copyright 2005, with permission from AOCS Press.

of 0.25–0.36 µm with CVs of 42–53%. Then, monodisperse W/O/W emulsions with diameters of 39.0–41.0 µm with CVs below 5% were formed by forcing the W/O emulsion into the external aqueous phase using the straight-through MC device.

21.4
Two-dimensional Microfluidic Systems

Two-dimensional (2D) microfluidic systems that can produce highly monodisperse emulsion droplets have been intensively studied in various fields [59–63]. Confined microfluidic channels such as T-junctions [64–68], cross-junctions [69–71], flow-focusing geometries [72–79] and other co-flow geometries [67, 80, 81] are generally used. Under the conditions of low Reynolds and capillary numbers [66], highly monodisperse emulsion droplets are reproducibly formed in the channels, typically

with CVs of 1–3%. The droplet size and breakup rate can be controlled precisely by changing the channel geometry, fluid properties and fluid speed. Channels with a hydrophobic surface are usually suitable for producing W/O droplets, whereas channels with a hydrophilic surface are used for producing O/W droplets. For example, polydimethylsiloxane (PDMS) [66, 68, 70–72, 78] and poly(methyl methacrylate) (PMMA) [65] can be used for the formation of aqueous droplets in an organic stream. O/W emulsion droplets can be formed in channels made of glass [67, 80, 81], silicon [69, 73], polyurethane [74–77], thiolene-based optical adhesive [79], etc.

Since the wettability of the channel determines the type of emulsions that can be formed, double emulsions can be produced in two consecutive microfluidic junctions each having opposite wetting properties [82–88]. For producing W/O/W droplets, for example, the combination of a hydrophobic upstream junction and hydrophilic downstream junction is needed (Figure 21.6a). At the upstream junction, aqueous droplets of uniform sizes that are to be encased are formed with regular periodicity in the middle organic stream. Then, in a continuing series, organic droplets that encapsulate aqueous droplets are formed in the external aqueous phase at the hydrophilic junction. By reversing the order of hydrophilic and hydrophobic junctions, O/W/O emulsions can be formed similarly. Both the size of internal and external droplets and the number of internal droplets can be precisely controlled by varying the flow rates of the three streams. For producing monodisperse capsules having an equal number of cores, two droplet-breakup rates at two junctions must

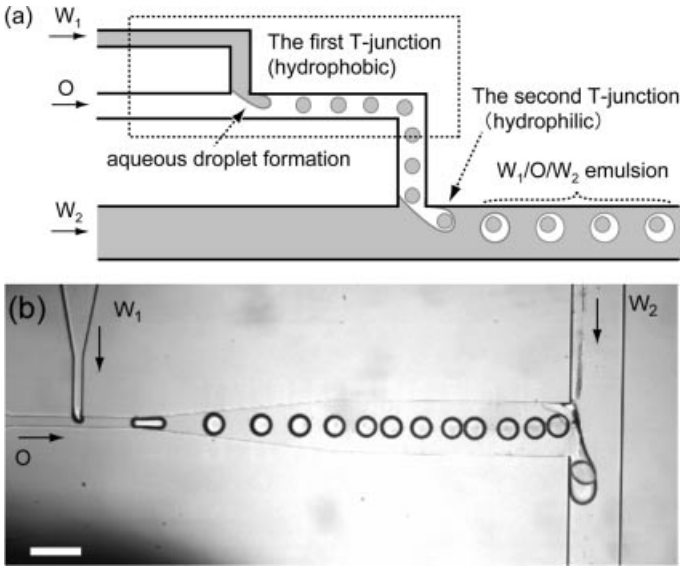

Figure 21.6 Controlled production of monodisperse double emulsions in a two consecutive microfluidic junctions [82, 83]. (a) Schematic illustration of two consecutive T-junctions for producing W/O/W droplets; (b) formation of W/O/W emulsion with single core in a glass microchannel. The channel has a uniform depth of 100 μm. Scale bar is 200 μm.

satisfy the following equation:

$$\frac{R_1}{R_2} = N \tag{21.2}$$

where R_1 is the breakup rate at the upstream junction, R_2 is the breakup rate at the second junction and N is a positive integer (1, 2, . . .). Small discrepancies in the two breakup rates cause a fluctuation in the number of internal droplets and the size of external droplets.

Figure 21.6b shows the formation of a W/O/W droplets through two consecutive T-junctions [82, 83]. An array of uniform aqueous droplets is formed within the organic stream at the hydrophobic upstream T-junction. Each aqueous droplet is then reliably encapsulated within an organic droplet at the next hydrophilic T-junction. Thus, highly monodisperse core–shell droplets with CVs below 3% can be formed reproducibly. By slight adjustment of the flow conditions, it is possible to control both the droplet size and the number of internal droplets precisely (Figure 21.7a–c). Moreover, it is also possible to control the compositions of the double emulsions by choosing an appropriate microfluidic geometry (Figure 21.7d).

A key question in this technique is how to pattern both hydrophilic and hydrophobic regions inside microchannels on a chip. One method is to prepare a hydrophobic section in a hydrophilic microchannel by localized surface modification [82–86]. This can be achieved by introducing reagents such as a silconizing fluid [82, 83, 85] and silane-coupling agents [84, 86, 89] into the channel networks. On the other hand, preparation of hydrophilic regions on a naturally hydrophobic surface is also possible. For example, Barbier *et al.* [87] reported the preparation of a stable hydrophilic section on a hydrophobic PDMS surface by deposition of a plasma-polymerized acrylic acid coating. They reported that the formation of a double emulsion using this hydrophilic surface is stable for more than 3 weeks, whereas PDMS treated with O_2 plasma became unstable within 1 day. Kumacheva and coworkers [88] also prepared hydrophilic regions in hydrophobic PDMS channels by using graft polymerization of acrylic acid.

Organic core–shell droplets in an aqueous phase, which can be described as an $O_1/O_2/W$ emulsion, can be produced in hydrophilic microfluidic channels [90–93]. In this case, localized surface modification is not needed when the organic phase to be encased (O_1) is more hydrophobic than the middle organic phase (O_2). For example, Kumacheva and coworkers [90] produced photopolymerizable droplets that encapsulate smaller droplets of silicone oil through the flow focusing of a coaxial stream of the two organic phases. Encapsulation of fluorinated oil droplets in other organic phases has also been reported [91–93].

21.5
Three-dimensional (3D) Coaxial Microcapillary Systems

Manufacturing equipment that consists of coaxial multiple nozzles is used commercially for the production of seamless multilayered microcapsules with diameters in

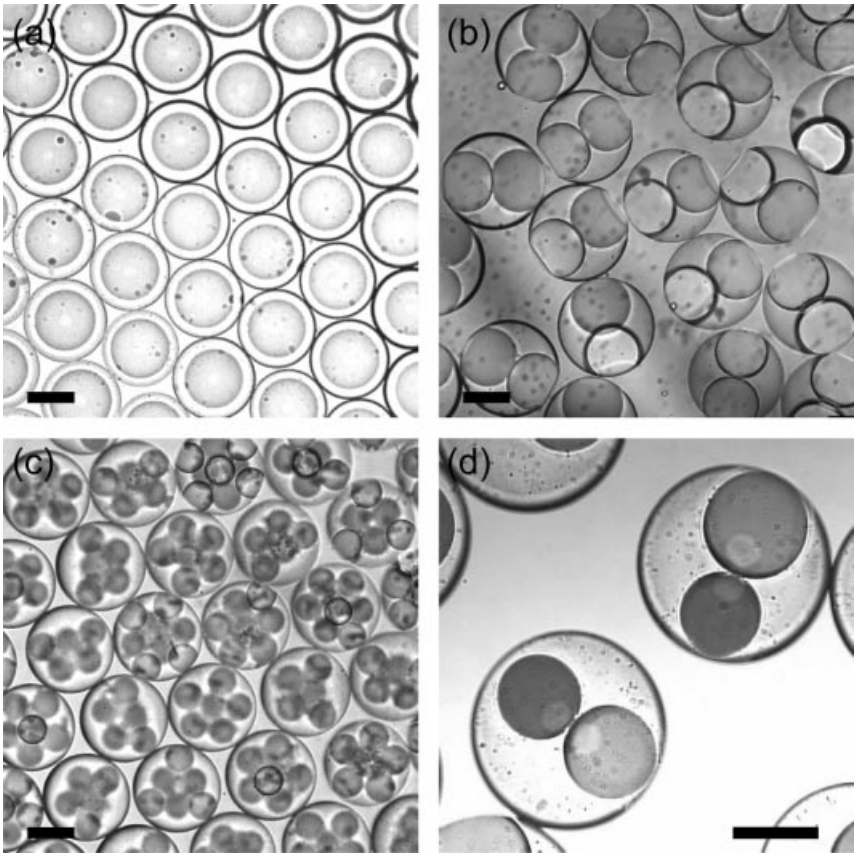

Figure 21.7 Monodisperse W/O/W emulsions with a controlled number of cores (*n*) and compositions [82, 83]. *n* = (a) 1; (b) 2; (c) 6–8. (d) Organic droplets with two aqueous cores from different sources. Scale bars are 100 μm.

the range 0.3–10.0 mm [94, 95]. Recently, similar systems consisting of concentric multiple microcapillaries have been studied for producing much smaller multi-layered emulsions with more complex internal structures.

A flow-focusing microcapillary platform [96–98] has been widely studied for the preparation of single- and multi-layered emulsions of uniform sizes. This technique is characterized by a narrow pinched geometry that hydrodynamically focuses a fluid stream to produce highly monodisperse droplets or bubbles. Figure 21.8a shows a microcapillary device developed by Weitz and coworkers [99–101] for producing highly monodisperse double emulsions. The device consists of three glass capillaries: two cylindrical inner tubes and an outer square tube lying in one axis. For producing O/W/O emulsions, the internal organic phase is pumped through a tapered injection tube and the middle aqueous phase is pumped through the outer region, forming a coaxial stream with the internal organic phase at the exit of injection capillary. The external organic phase is pumped through the outer region from the opposite

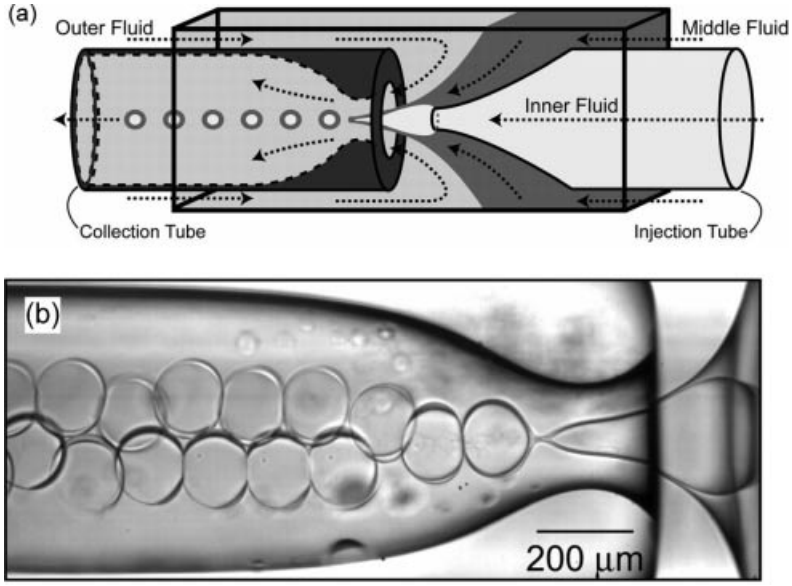

Figure 21.8 A microcapillary device for generating monodisperse double emulsion. (a) A schematic of the device and flow orientation; (b) formation of monodisperse O/W/O emulsion consisting of uniform water droplets having a single silicone oil droplet. CV is less than 1%. From Ref. [99]. Reprinted with permission from AAAS.

direction and the three fluids are forced into the collection capillary, breaking up into highly monodisperse O/W/O emulsion droplets (Figure 21.8b). The capillary geometry and relative flow rates can be varied to adjust the droplet size and the number of encased droplets. Similar axisymmetric coaxial flow-focusing devices are prepared by inserting micropipettes into a PDMS slab [102, 103] or fabrication of embedded orifices in SU-8 resist through layer-by-layer photolithography [104].

A distinct advantage of the coaxial capillary approach is in the surface treatment of the device; both W/O/W and O/W/O emulsions can be formed without any surface modification of the capillaries, because neither the middle nor the inner fluid touches the capillary wall surface. This is clearly different from the other techniques mentioned above in this section. An additional advantage is the flexibility of the system. For example, the structure of the device can be easily modified to make higher order multiple emulsions, such as triple emulsions [100, 101].

The use of the electrified jetting technique has also been reported for producing core–shell droplets and particles [105]. Two immiscible fluids are introduced at appropriate flow rates through two concentrically located needles. Then, by applying suitable voltages, a structured Taylor cone is formed and a steady jet that consists of compound droplets is generated. The size of the core–shell droplets can be varied in the range from sub-micrometers to 10 μm by changing experimental parameters such as flow rate, applied voltage and capillary diameter. The formation of polymeric shells with an internal aqueous phase has also been reported.

21.6
Applications to Novel Materials

There are a myriad of potential applications of highly monodisperse multiple emulsions with precisely controlled sizes and internal structures. Many new applications have recently been proposed by several groups. For example, Weitz and coworkers have reported various particulate materials synthesized from monodisperse double emulsions produced by their coaxial microcapillary devices. Examples include polymeric shells cured by UV irradiation [99], polymerosomes made from diblock copolymers [99, 106, 107] and shells of liquid crystals with novel defect structures [108]. They also produced thermosensitive microcapsules from a W/O/W/O triple emulsion, affording pulsed release of the innermost aqueous droplets by elevating the temperature of the environment [101]. Kumacheva and coworkers [90] produced polymeric microparticles with tunable morphologies from $O_1/O_2/W$ emulsions prepared in the 2D coaxial flow-focusing geometry. Other new materials reported thus far include polymeric shells with a pore on their surfaces [91, 92], templated silica particles synthesized from an O/W/O emulsion [85] and acoustically active dual-layer lipospheres for drug delivery made from a $gas/O_1/O_2$ emulsion [109].

The industrial production of these new materials would require drastic scale-up of the productivity, because the throughput in each microstructured device is very low, typically below a few grams per hour. One promising solution to this issue might be the large-scale parallelization of the 2D microfluidic channels on a chip, which has been successfully demonstrated for the mass production of monodisperse single emulsions and polymeric microparticles [110].

21.7
Conclusion

In this chapter, an overview is provided of new emerging technologies for the preparation of monodisperse emulsions with complex multilayered structures. The droplet size, internal structure, and even the compositions of multilayered emulsions can be flexibly controlled by using various microstructured devices. Since each emulsification technology has its own characteristics, the combination of different technologies might enhance the type of multilayered emulsions that can be prepared.

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