19
Microencapsulates, Proteins and Lipids/Vesicles

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19.1
Introduction

Polymeric microencapsulates and lipid microencapsulates have extensive potential applications in food, cosmetics and pharmaceutics [1–5]. Microencapsulates can protect and conserve an active component until its release is desired and stimulated. Polymeric microencapsulates consist of a (biocompatible) polymer matrix in which an active component is encapsulated. Most frequently poly(lactic acid) (PLA) or poly (lactic-co-glycolic acid) (PLGA) is used as the polymer [6, 7], but alternatives have been investigated [8, 9]. Lipid microencapsulates, lipid vesicles and liposomes are composed of a (phospho-)lipid bilayer membrane that encapsulates an aqueous volume, thus mimicking a cell structure.

In general, polymeric microencapsulates are formed from a dispersion of an organic solution in water. The organic solution usually consists of dichloromethane (DCM), a polymer that will form the solid matrix of the particle, the active component and optionally some surface-active species. If the active component cannot be dissolved in the organic solvent, a double emulsion is made, and the droplet with organic solution then contains smaller aqueous droplet(s) which contain the active component. In a consecutive step, the organic solvent is removed through extraction and/or evaporation, rendering the solid polymer microcapsule loaded with active component. Liposomes are produced similarly from a double emulsion.

Polymeric microencapsulates and liposomes allow the controlled release of drugs and enzymes or proteins [10–23] and dyes [24–26]. Applications of liposomes in biochemistry also include interrogation [27–31], perturbation [32–36] and stimulation [37–41] of the cellular environment. The control of the size and content of microencapsulates is of critical importance for successful application in targeted drug delivery and transfection of DNA, RNA, protein or antibodies [42–44]. The size determines the rate at which the phagocytes in the (human) body break down the microencapsulates and thus determines the drug dosage. Microfluidic systems allow process control at the desired micro- and nanoscale, which cannot be obtained with conventional bulk production methods. The laminar flow conditions in microfluidic

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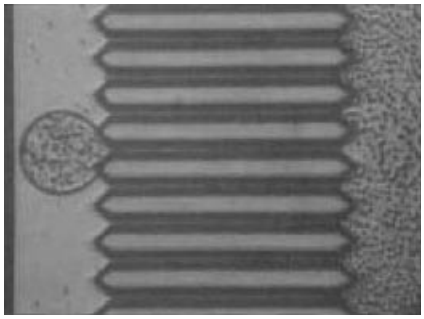

Figure 19.1 Formation of a double emulsion droplet (left) from a single emulsion (right). From Kawakatsu *et al*. [47].

channels create a well-defined and stable interface between two fluids and can be used to focus fluid streams to submicron dimensions for rapid mixing and patterning [45, 46].

19.2 Production Methods

Kawakatsu *et al*. [47] produced water-in-oil-in-water (W/O/W) emulsions by microchannel emulsification of a water-in-oil (W/O) emulsion in water (Figure 19.1). The W/O emulsion was prepared by homogenization. Polyoxyethylene sorbitan monolaurate (Tween 20) was used in the external water phase to stabilize oil droplets containing water droplets. Sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80) and tetraglycerol polyricinoleate (TGPR) were tested as surfactants to stabilize the feed W/O emulsions. W/O/W emulsions were produced when using oleic acid or triolein as the oil phase and TGPR as the surfactant, while W/O/W emulsions could not be produced using Span 20 or Span 80 due to large water clusters and the low stability of the W/O emulsions. Using oleic acid as an oil phase, monodisperse W/O/W emulsions were obtained, while polydisperse W/O/W emulsions were produced using triolein as the oil phase, probably due to the low production rate of the emulsion and fluctuation of the production rate. The concentration of TGPR affected the stability of the internal water droplets and oil droplets containing the water droplets. At a high TGPR concentration, the internal water phase was stable; however, oil droplets containing water droplets had a tendency to coalesce. W/O/W emulsions that contained pectin solution as an internal water phase were also produced using oleic acid and TGPR as the oil phase and surfactant in the oil phase, respectively. Solid-in-oil-in-water (S/O/W) pectin microcapsules were obtained by gelation of the internal water phase using an external water phase with a calcium solution containing Tween 20.

Abraham *et al*. [48] fabricated spherical polymeric microcapsules by flow focusing of an organic phase consisting of amphiphilic block copolymer solution in DCM with a continuous aqueous phase in a cross-junction microchannel of $100 \times 100\,\mu m$ channel cross-section. The ratio of the flows of the two immiscible solutions

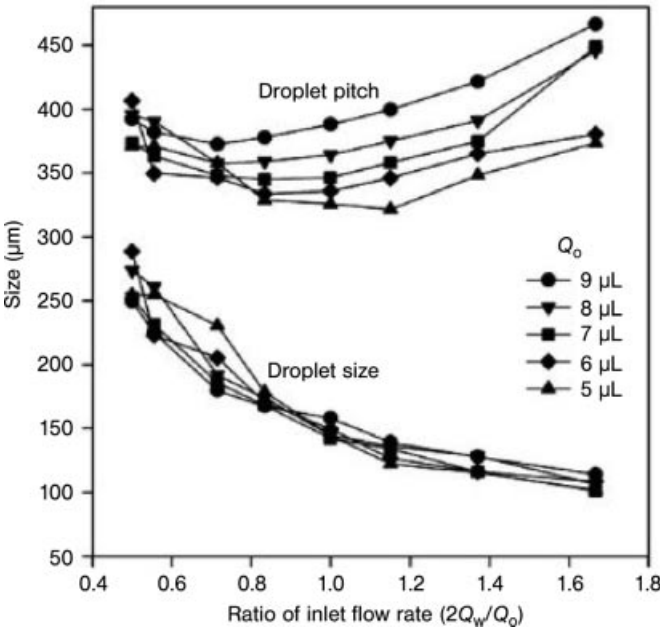

Figure 19.2 Droplet size and pitch as a function of organic/aqueous flow rate ratio. From Abraham *et al.* [48].

determines the size of the organic phase droplets. The droplet diameter ranged from $270 \pm 20\,\mu m$ at a W/O flow ratio of 0.5 to $110 \pm 10\,\mu m$ at a W/O flow ratio of 1.7 (Figure 19.2). The organic phase flow rate ranged from 5 to 9 $\mu L\,min^{-1}$; no significant influence on droplet diameter was observed. The evaporation of DCM resulted in solid microcapsules with a diameter of 50% of the original droplet size. Morphological studies showed that the microcapsules had a hollow inner cavity and a porous shell. The efficiency of these polymer microcapsules as containers for the storage and controlled release of loaded molecules was evaluated by loading the microcapsules in a consecutive step with Congo Red. The release performance was evaluated at various pH levels and temperatures with UV–VIS spectroscopy. The release performance of the microcapsule was found to depend on the swelling of the microcapsule in the solution and the diffusivity of the dye.

Thorsen *et al.* [49] investigated droplet formation at a T-junction. The measured channel dimensions are approximately 60 µm wide and 9 µm deep, tapering to 35 µm wide and 6.5 µm deep in the region where the water and oil–surfactant mixture meet at the crossflow intersection. The water flow was injected perpendicular to the oil flow. The actual oil and water flows are not given, only the driving pressures. At fixed oil pressure, the droplet size increases with water pressure, ranging between 10 µm (low water pressure, corresponding to low water flow)and 40 µm (high water pressure corresponding to high water flow). Because of the dependence of the oil flow on total pressure drop, the increase in water pressure would have resulted not only in an increase in water flow but also in a decrease in the oil flow. On expansion of the flow

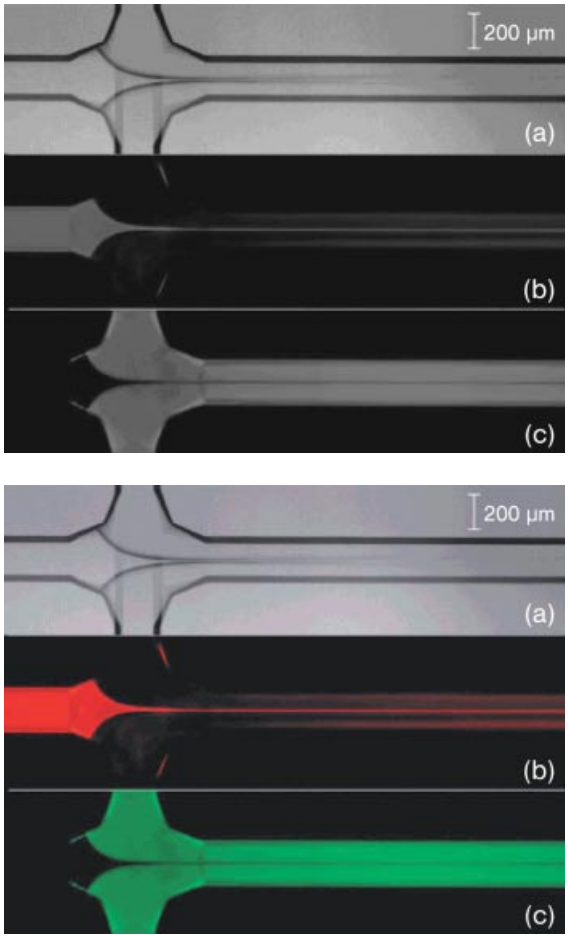

Figure 19.3 (a) White light image of hydrodynamic focusing of IPA by buffer streams. Fluorescence images of (b) DiIC18 in IPA stream and (c) CF in buffer streams [51].

from 35 to 60 µm, they observed different patterns of droplets, a solid water stream, elongated droplets, triple droplet layer, double droplet layer, jointed droplets and separated droplets. Without exact information on mass flows of the oil and water phase, these results are difficult to convert into a device-independent flow map. Quevedo *et al.* [50] used simple PVC tubing of 1.6 mm i.d. and a needle of 160 µm i.d. to mimic a microfluidic system; the immiscible solutions were introduced into the device by two separate syringe pumps. Laminar, transitional, droplet and chaotic phases were observed, similarly to classic microfluidic devices. At an organic flow rate of 0.141 mL min^{-1} the droplet diameter was 865 µm at 2.0 mL min^{-1} and decreased to 313 µm at 25 mL min^{-1} (SD of droplets not given).

Jahn *et al.* [51] use flow focusing in a cross-junction for the spontaneous self-assembly of liposomes from a solution of dissolved phospholipids. An organic phase

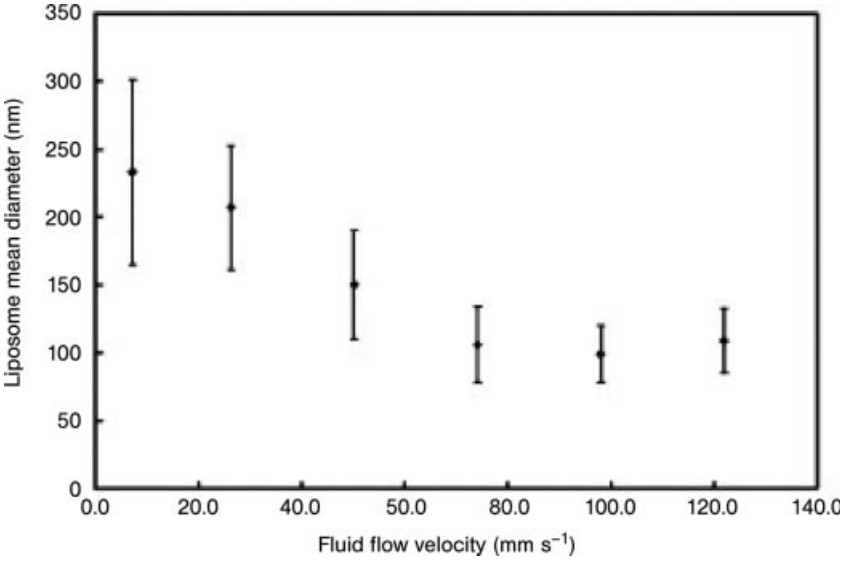

Figure 19.4 Liposome mean diameter measured by light scattering versus the combined fluid flow velocities of all inlets. IPA inlet velocity is 2.4 mm s^{-1} [51].

is focused at a microchannel cross-junction between two aqueous buffer streams (Figure 19.3). As organic phase, isopropyl alcohol (IPA) containing the dissolved lipids and a fluorescent dye (DiIC18) flows through the center inlet channel and an aqueous phosphate-buffered saline solution flows through the two side-inlet channels. A sheet is formed from which nanosized droplets shear off. At an organic phase velocity of 2.4 mm s^{-1}, the droplet diameter is 240 ± 60 nm at 4.8 mm s^{-1} aqueous phase flow and levels off at a droplet diameter of 100 ± -20 nm at 70 mm s^{-1} and higher (up to 120 mm s^{-1}) (Figure 19.4).

Wischke and coworkers [52, 53] use a static interdigital mixer to formulate poly (lactide-co-glycolide) (PLGA) microparticles using a W1/O/W2 solvent evaporation procedure, in order to obtain suitable vehicles for vaccination. In a first step, primary emulsion of water with active component in dichloromethane was made. In a second step, the primary W1/O emulsion was emulsified with the W2 phase in an interdigital mixer (Figure 19.5). The influence of different techniques for the preparation of the primary W1/O emulsion was investigated with respect to the active component localization within the microparticles, morphological characteristics of these particles and active component release. A rotor–stator homogenizer resulted in a primary emulsion with droplet diameters of 2.5 ± 1.1 µm. The secondary emulsification step led to a broad and asymmetric particle size distribution for all flow rates investigated. At 5 mL min^{-1} the average particle diameter equals 35 µm (SD not given), which levels off to a value of 3 ± 1 µm at 25 mL min^{-1} (much larger particles are still visible on the SEM pictures, however). The capture efficiency of active component is independent of flow rate and is 75%. Solvent evaporation resulted in hollow

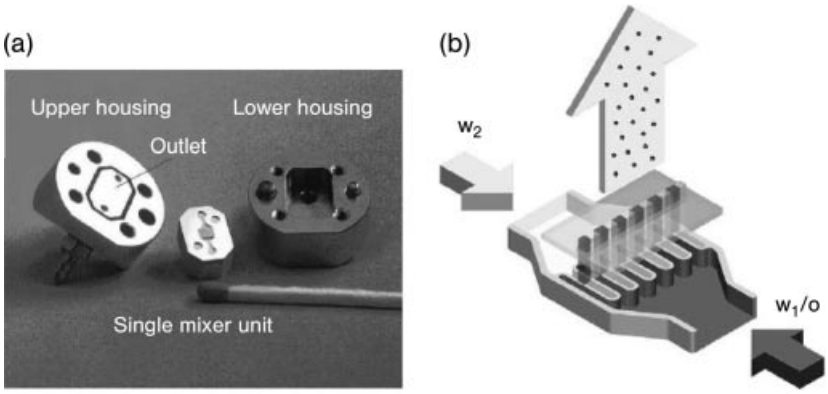

Figure 19.5 Construction and mode of operation of the standard slit interdigital micromixer. (a) Left, upper housing with two inlets and the outlet slit in the middle; center, inlay with interdigital mixing structure; right, lower housing with cutout for the inlay. (b) Mixing principle: fluids to be mixed flow into mixing channels, formation of a fluid lamellae and disintegration into droplets [53].

particles with one pore with a high burst release, because nearly all active component was attached to the outside of the particle wall. A high-pressure homogenizer and an ultrasonic procedure gave primary W1/O emulsions with droplet sizes of $0.57 \pm 0.09\,\mu m$ and lower, which resulted in solid particles and a uniform distribution of active component. This uniform distribution resulted in a slower release of active component. With the same procedure, the authors prepared physically stable cationic microparticles for protein delivery to dendritic cells, which might be used for dendritic-cell based therapies [54].

Freitas and coworkers [55, 56] developed a flow-through ultrasonic cell for the preparation of the primary emulsion. A steel jacket is excited with an ultrasonic transducer, which transmits the sound waves via pressurized water to a glass tube installed inside the jacket. The authors combined this ultrasonic cell combined with a static interdigital micromixer for the production of the double emulsion. Microspheres were prepared from poly(lactic-co-glycolic acid) (PLGA) and bovine serum albumin (BSA) served as a model protein for microencapsulation. The BSA-in-PLGA (W/O) emulsions produced by the ultrasonic flow through cell exhibited mean droplet sizes of less than 700 nm. Further processing into microspheres of 15–40 μm mean diameter resulted in ~70% BSA encapsulation efficiency with excellent batch-to-batch reproducibility. The setup allows for relatively easy aseptic microsphere production. The product sterility was confirmed by a simple sterility test.

Tan *et al.* [57] developed a method for encapsulating biological species ranging from tens of microns-sized cancer cells, microsized fluorescent beads, to nanosized protein molecules in a single-step process using a droplet based microfluidic system to control the encapsulation efficiency. They used flow focusing in a cross-junction to make an emulsion of water in oil. The water phase contains an active component, i.e. live cells, micro-/nanobeads or protein molecules. In a second step, with a syringe,

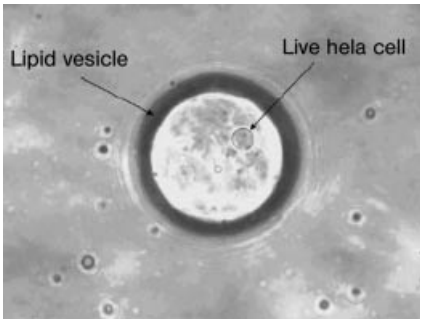

Figure 19.6 A live HeLa cell encapsulated in a lipid vesicle [57].

this emulsion is added to a water–ethanol mixture for the extraction of the organic phase, rendering the desired lipid vesicles of $27 \pm 6\,\mu m$ for the protein molecules and $60 \pm 12\,\mu m$ for the micro-/nanobeads and the cells (Figure 19.6). Tan *et al.* determined that the vesicle yield and the encapsulation efficiency depend both on the continuous flow rate applied to the microfluidic system and on the concentration of ethanol contained in the dissolution mixture. A lipid emulsion generated with a $1\,\mu L\,min^{-1}$ lipid flow rate produced large droplets (>78 μm) with beads entrapped in 85% of the droplets but only yielded 5% of beads entrapped in the final vesicles due to breakage in the second step. Smaller droplets (<55 μm) generated with 7–9 $\mu L\,min^{-1}$ lipid flow rate limits the bead encapsulation efficiency to 15% but yields a bead encapsulation in vesicles from 64% to 91%.

Tong *et al.* [58] investigated the formation of monodisperse oil-in-water (O/W) microencapsulates using lecithin and lysophosphatidylcholine (LPC) as surfactant by applying a microchannel emulsification technique. Lecithin led to the coalescence of the microencapsulates formed and the continuous outflow of the oil phase through the microchannel, due to the insufficient interfacial activity of lecithin and the subsequent wetting of the microchannel surface by the oil phase during the emulsification process. Monodisperse O/W microencapsulates were produced by using lecithin and hydrophilic LPC dissolved in the water phase but not with lecithin as the only surfactant. Also, a more stable emulsification process producing monodisperse O/W microencapsulates was found using lecithin in the oil phase and LPC in the water phase. The monodisperse O/W microencapsulate production was improved by a special surface oxidation treatment of the microchannel plate. The observed droplet diameters were 28.5 ± 0.4 and $30 \pm 0.7\,\mu m$ for the 8.9 and 5.8 μm channel diameters, respectively. The flow rates are not given explicitly; a flow rate of two drops per second per channel ($1.7 \times 10^{-3}\,\mu L\,min^{-1}$) is observed at 0.4 kPa total pressure drop in the 8.9 μm channel diameter. In total, 600 channels were operated adjacently on the microchannel plate.

Yang *et al.* [59] generated monodisperse tripolyphosphate (TPP)–chitosan microparticles using a cross-flow microfluidic chip coupled with external crosslinking reaction. They controlled the size of TPP–chitosan emulsions from 180 to 680 μm in diameter (with a variation of less than 10%) by flow focusing and changing the liquid flow rate ratio. This emulsion, consisting of aqueous 1% w/v chitosan, is

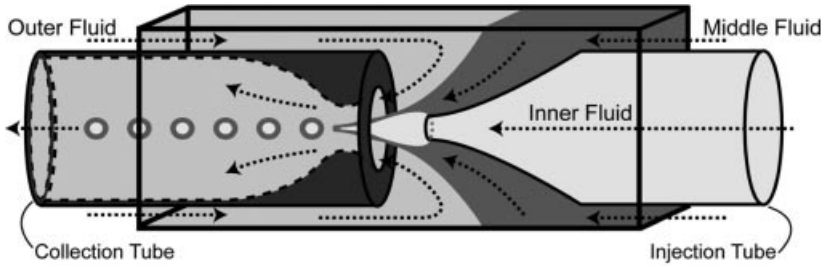

Figure 19.7 3D microfluidic device for creation of double emulsions [60].

consecutively dripped into a solution containing 10% w/v TPP. An ionic cross-linking reaction then creates water-insoluble TPP–chitosan microparticles. The size distribution of the resulting TPP–chitosan microspheres is narrow (not specified), important for an optimal release rate in the administration of controlled-release drugs. The proposed microfluidic chip is capable of generating relatively uniform micro-droplets and has the advantages of actively controlling the droplet diameter and having a simple and low-cost process, with a high throughput.

One future prospect for microfluidic control will be the development of tailor-made vesicles with more complex geometries, triple, quadruple, multiple emulsions and artificial cells (artificiells), i.e. liposomes containing liposomes with each a different functionality, closely mimicking a biological cell structure, all designed for optimal performance for the specific function demanded from the vesicle. Double emulsions had already been made in a microfluidic device in a very controlled way [60, 61] (Figures 19.7 and 19.8). The number of secondary droplets inside the primary droplet

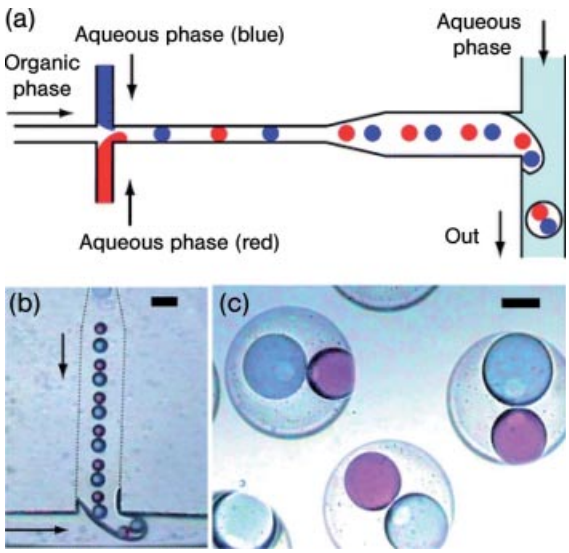

Figure 19.8 Microfluidic device for the production of a multiple double emulsion [61].

can be varied and, by introducing water phases of different composition, even a multiple double emulsion can be made, in which the primary droplet contains two different species of secondary droplets. Engl and coworkers [62, 63] gave an outlook on the possibilities that this gives, but did not report any relevant results.

19.3 Conclusion

Polymeric microencapsulates and lipid microencapsulates have extensive potential applications in the food, cosmetic and pharmaceutical markets. Successful introduction in these markets heavily depends on the control over the production process. The droplet size and the droplet size distribution of internal and external phases determine the product quality, e.g. the release rate of active ingredient. The capture efficiency of the active ingredient determines the process economy. The literature on the production of microencapsulates is on a trial and error basis. No general rules have been developed that can be used to design a microfluidic chip for any combination of aqueous and organic phase. Future research should focus on the development of these design rules; what is the influence of channel dimensions and liquid properties on the droplet formation. Furthermore, research should focus on the scale-up of the production process without losing control over the droplet formation; the reported production rates of single microfluidic channels is unsatisfactory for the food, cosmetic, and pharmaceutical markets.

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