Microchannel Emulsification Processes for Monodisperse Water-in-Oil Emulsions

July 2017

Ran LI

Microchannel Emulsification Processes for Monodisperse Water-in-Oil Emulsions

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

The University of Tsukuba

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy in Bioresource Engineering

(Doctor's Program in Appropriate Technology and Sciences for Sustainable Development)

Ran LI

Contents

Nomenclature

CV	coefficient of variation [-]
$d_{n,drop}$	number-weighted mean droplet diameter [μm]
d_{drop}	droplet diameter [μm]
g	acceleration due to gravity [m/s^2]
ΔP_d	pressure applied to a dispersed phase [Pa]
Δh_d	height of a dispersed-phase chamber [μm]

Greek symbols

θ_d	contact angle of a dispersed phase [deg]
θ_w	water contact angle on microchannel plate [deg]
γ_L	liquid surface free energy [J/m^2]
γ_S	solid surface free energy [J/m^2]
γ_{SL}	liquid/solid interfacial free energy [J/m^2]
ζ	zeta potential of microchannel plate surface [V]
σ	standard deviation [μm]

Chapter 1
Introduction

1.1 Background

Water-in-oil (W/O) emulsions play an important role in many fields, e.g. food, chemical, and pharmaceutical industries. There are some W/O emulsion-based food products, such as butter and margarine. The properties of W/O emulsion will affect the quality of food products. The droplet size and size distribution are the most important parameters, which can determine the stability, chemical reactivity, and physiological efficiency of W/O emulsion (Khalid *et al.,* 2016). If the W/O emulsion is polydisperse, the droplets are more susceptible to occur coalescence, which will influence the taste and shelf time of food based on W/O emulsion. Therefore, to prepare monodisperse W/O emulsions has been considered.

W/O droplets with the size range of 50-1000 μm normally used as templates for value-added products which can be formulated into microcapsules, and microparticles via polymerization, gelation, and other processes in food industry. In addition, the monodispersed W/O emulsions with large droplet size are good templates for micron-scale biological materials because of the high potential controlling on the release of bioactive substances yielded contained in micromaterials.

In practice, the W/O emulsions prepared by conventional devices, e.g. high-pressure homogenization, rotor-stator systems (e.g. stirred vessels and colloid mills), and ultrasonic homogenizersare usually polydispersed having a coefficient of variation (CV) >20% (Kawakatsu *et al*., 2002). In the past two decades, some novel devices, such as membraneemulsification, microfluidic emulsification and direct microchannel emulsification have been proposed to prepare monodisperse W/O emulsions. However, a

major disadvantage of these devices is that they have lower productivity than conventional devices. Therefore, to prepare monodisperse W/O emulsions for large-scale production is considerable.

1.2 Emulsions

1.2.1 Definition of emulsion

Emulsions are widely applied in various fields, such as food, chemical industries, pharmaceutical or cosmetic. An emulsion is a combination of two or more liquid phases which are normally immiscible, that is made of at least one phase dispersed in another. An emulsion formed by homogenizing pure water phase and oil phase together is unstable since these two phase will soon separate into two layers, with the oil layer being on the top of the water layer since of the different density. Emulsifiers can reduce the interfacial tension and stabilize emulsions by diminishing the repulsive force between droplets. The formulation of an emulsion contains the time of the emulsifier molecules to organize at the interface of two phases and the mechanical mixing of the immiscible phases (McClements, 2004). The average diameter of emulsion droplets is usually ranging from 0.1 μm to 100 μm. There are many typical emulsion types classified according to the distribution of the water phase and oil phase. As shown in Figure 1-1a, the emulsion is combined by water phase dispersed in the oil phase that is defined water-in-oil (W/O) emulsion. By contrast, the emulsion which is combined by oil phase dispersed in the water phase is called oil-in-water (O/W) emulsion shown in Figure 1-1b. These single emulsions are also commonly

prepared as a dispersed phase to produce double emulsions, such as water-in-oil-in-water (W/O/W) emulsion and oil-water-in-oil (O/W/O) emulsion shown in Figure 1-1c and d.

1.2.2 Droplets size and size distribution

There are many important properties of emulsion-based food produces are affected by droplet size and its distribution, such as appearance, shelf life, texture, and flavor. First of all, the emulsion droplet size can affect the appearance and taste of products. The emulsion droplets can diffusely reflect light, which resulted in the emulsion having a whitish color. For example, when the emulsion droplet size is larger, the emulsion will be more opaque and whitish. By contrast, when the emulsion droplet size is smaller, the emulsion will be more transparent. However, when the emulsion with droplet size < 100 nm, the emulsion will be opaque or transparent. An O/W food emulsion which contained smaller sized droplets suppresses bitterness (Nakaya *et al.*, 2016). The droplet size distribution has effect on many chemical, physical and biological properties of the emulsion (Kobayashi *et al.*, 2012). The emulsion with wide droplet size distribution are usually polydisperse which are easier to occur coalesce than the emulsion with narrow droplet size distribution (Saito *et al.*, 2006). Some food products based on W/O emulsions e.g. margarine always add flavoring into the water phase which flavor may affected by the droplet size distribution. In addition, the narrow droplet size distributions are resulted in larger total surface area of emulsion droplets than emulsion with wide droplet size distribution, which has benefit for components released from the surface. The droplet size distribution also can affect the growing of bacteria. The bacteria need enough space to grow and absorb nutrients from the

droplets. When the droplet size is smaller, the bacteria cannot get sufficient space and nutrients inside the droplet which can greatly inhibit bacteria growth. The emulsion will separate into two or more phases which is influenced by many factors, such as coalescence, creaming, sedimentation, and flocculation. Creaming is a phenomenon that the oil droplets float up to the O/W emulsion surface, and sedimentation is the aqueous settle down the bottom of the W/O emulsion. Both of these phenomenon are driven by the different density between continuous phase and dispersed phase. Broader droplet size distribution in an emulsion becomes easier to cause creaming or sedimentation (Saito *et al.*, 2006). Therefore, it is helpful to know more information about the droplet size and its distribution in an emulsion.

1.2.3 Monodisperse emulsions

Emulsion stability is a very important factor to value the quality and shelf-life of emulsion used in food industry. The coefficient of variation (CV) that a standard deviation depended on the droplet size, is normally used to express the droplet size distribution. If the CV of mean emulsion is above 25%, the emulsion is polydispersed. By contrast, the monodisperse emulsion has a CV < 25%.Monodisperse emulsion has better controllability in the terms of stability and properties. There are some methods to improve the stability of emulsion. One is to prepare monodisperse emulsion with smaller droplet size and narrower droplet size distribution. Monodisperse emulsion is good for fundamental research because of the better stability than polydisperse emulsion (McClements, 2004). The droplets in monodisperse are much simple which mean the changes of droplet size can be easier

observed. Furthermore, monodisperse emulsion with better stability also has potential applications in many fields to prepare emulsion-based products, e.g. microparticles, microcapsules, and giant vesicles (McClements, 2004).

1.2.4 Application of W/O emulsion

Emulsions play an important role in the formulation of food industries. O/W emulsions are usually used to prepare food emulsions including mayonnaise, artificial milk, soups, dressing, low fat spreads and sauces. W/O emulsions are normally used to produce food emulsions, e.g. butter and margarine. Some emulsions are the finished food products in themselves, such as cream liqueurs, ice creamers and coffee creamers that are simple emulsions remained stable occurs coalescence and creaming during their shelf-life and production. W/O emulsions also can be used to prepare W/O/W food emulsions which has been growing interest in complex food products because of the potential benefits, e.g. controlled, reduction of the total fat content, protection of labile ingredients that might normally interact with each other.

As stated above, the most important factors for stability and properties of W/O emulsion-based food products are droplet size and droplet size distribution. However, it is difficult to produce monodisperse W/O emulsion by using conventional emulsification devices. Some newly emulsification devices have been developed to produce monodisperse W/O emulsion in the past twenty years. We will discuss the major emulsification devices later.

1.3 Emulsification devices

1.3.1 Conventional emulsification devices

As emulsion is a thermodynamically system, emulsification requires external energy to the emulsion system. In general, emusification is started by gently mixing of the dispersed phase and continuous phase to obtain premix emulsion. The following step is to use homogenization which can apply forces to break up the droplet of premix emulsion into smaller size. The progress of homogenization is intense to disrupt or combine the premix droplets, whose forces is larger than the interfacial tension forces between dispersed phase and continuous phase that can keep the droplets closer to break up into smaller size. In order to decrease the energy input of emulsification, reduction of the interfacial tension is needed. Emulsifier consists of hydrophobic and hydrophilic molecule, which can absorb on the droplets surface to lower the interfacial tension (Shima, 1998). In addition, the higher adsorption rate of emulsifier, the faster reducing of interfacial tension. Thus, the selection of emulsifier for an emulsion system is important.

Several types of conventional emulsifications are used into industrial productions. Rotor-stator homogenization (Figure 1-2a), utilizes mechanical stirring or shearing to break up the large droplets into smaller ones. The typical droplet size prepared by rotor-stator ranges from 1 μm to 30 μm. The droplet size distribution which defend by CV is greater than 30%. The largest production of Rotor-stator is around 100 L/h. A disadvantage of this technique is dissipation of most of the energy input the emulsion system, causing temperature elevation.

High-pressure homogenization (Figure 1-2b), injects the premix emulsion through small orifices where high extension and shear force are applied to break up them into smaller droplets. High-pressure homogenization can prepare droplets with sizes ranging from 0.05 μm to 2 μm. The CV of result emulsion is usually > 20%. The maximum productivity of this system is about 10000 L/h. However, a drawback of this technique is that the manufacturing cost is high due to the low energy efficiency of high-pressure homogenization.

Ultrasound emulsification shown in Figure 1-2c is also one of the commonly used techniques. It can be emulsificated by an ultrasonic to place the premix in a vessel. This technique is driven by ultrasound waves to break up the premix emulsion into smaller droplets. Ultrasound emulsification is able to produce emulsion with droplet sizes around 1 μm to 30 μm. The CV of emulsion is typically >30%, and the maximum productivity is up to 1000 L/h. This mothed is suitable to prepare emulsion with small volume for lab research.

All of the methods described above are energy inefficient. The energy input is much higher than necessary requirement in an emulsion system, only 1-10% of all input energy is used for droplet break-up. In addition, the droplet size distribution of emulsion prepared by these techniques is normally wide.

1.3.2 Membrane emulsification

In the last two decades, many new emulsification devices have been developed to prepare monodisperse emulsions. Membrane emulsification has attracted an increasing interest in

various industries, e.g. foods, chemicals, pharmaceutics, and cosmetics. In general, there are two membrane emulsification methods. The first one is direct membrane emulsification in which the dispersed phase is forced to pass through a membrane with a uniform pore-size distribution into the continuous phase using a low pressure (Figure 1-3a). In the direct membrane emulsification process, the continuous phase covers on the membrane surface, while the dispersed phase passing through the pores to meet the continuous phase (Schröder *et al.,* 1998). The droplet gradually grows and will detach when reaching the critical size at the outlets of pore. The critical size is determined several factors, e.g. the interfacial tension, the operating pressure, and the balance between the drag force by the flowing continuous phase and disperse phase. Both the pore size on membrane and the degree of coalescence which depending on the continuous phase flow rate and the contact angle between the surface membrane and droplet, are able to determine the final droplet size and size distribution (Peng *et al.,* 1998). Direct membrane emulsification can prepare droplets with sizes of 0.3 μm to 100 μm. The CV of resultant emulsion is around 10% - 30%. One of the advantages for this method is that the droplet size can be controlled primarily by selection the membrane pore size rather than the preparation of turbulent droplet breakup. The lower energy requirement and less amount of emulsifier than conventional methods are also benefits for producing emulsions. Direct membrane emulsification is applicable to prepare both W/O emulsion and O/W emulsion by hydrophobic and hydrophilic membranes, respectively (Suzuki, *et al.,* 1996). The maximum flux of dispersed phase in direct membrane emulsification is reported to be 2.5 m^3/m^2h. Due to the low disperse phase flow rate, direct membrane emulsification is suitable for preparing monodisperse

emulsions with low volume fraction of the dispersed phase, typically up to 25 vol% (Katoh *et al.*, 1998; 2004).

Another method of membrane emulsification is called premix membrane emulsification (Figure1-3b). Premix membrane emulsification is based on the direct membrane emulsification. This method contains two steps: the first step is to prepare a polydisperse premix emulsion, driven by a gentle homogenization. The second step is to inject the premix emulsion through the membrane pores to break up into smaller droplets with a narrow size distribution. In order to obtain final fine emulsion with narrow droplet size distribution, the premix can pass through the membrane pore repeatedly (Vladisavljević *et al.*, 2004). There are several advantages of premix membrane emulsification compared with direct membrane emulsification. The volume fraction of dispersed phase is much higher than in direct membrane emulsification, leading to the flux of dispersed phase in premix membrane emulsification being than 1 $m^3/(m^2\ h)$. This advantage means premix membrane emulsification can be used for preparing large-scale monodisperse emulsions. The final droplet size is smaller than that in direct membrane emulsification.The resulting droplet size can be easier controlled by premix emulsification than direct emulsification.The setup used for premix membrane emulsification is simpler, since it does not need moving parts, e.g. stirrer or pump during emulsification.

1.3.3 Microfluidic emulsification

Microfluidic emulsification has been paid increasing attention to applications in chemical, biology, medicine, and food industries. Microfluidic emulsification can prepare simple or

multiple emulsions including structured outer or inner droplets, which has potential food applications as value-added products. The continuous phase and dispersed phase flowing in each channels, are combined at a T-, Y-junction to form droplets. Figure 1-4a and b shows two representation types of microfluidic emulsifications: T- and Y- junctions. At the beginning of this process, the dispersed phaseand continuous phase individually flow in each channel, and then the dispersed phase starts into the channel for the continuous phase at the junction to form a droplet, the droplet will be detached and flow into the channel which full of continuous phase. This droplets detached process is driven by the continuous phase flowing in the channel. There is a different another type of microfluidic emulsification called microfluidic flow focusing device (Figure 1-4c), the continuous phase and dispersed phase pass through a small pore in the downstream of channels. The dispersed phase will flow into a narrow thread driven by a low pressure and shear stress provided from the flowing continuous phase. The droplet size and size distribution are mainly affected by the pore or nozzle size. The CV of resulting emulsion is always below 5%. The size of droplets prepared by these microfluidic devices rangs from 5 μm to 30 μm with the maximum productivity of 0.3 L/h (Vladisavljević *et al.,* 2012; Kobayashi *et al.,* 2012). In this emulsification, W/O emulsion and O/W emulsion can be prepared depending on the wetibility of channel. When the channels are formed by hydrophobic material, it is suitable to disperse aqueous droplets in oil phase. In contrast, if the MCs are formed by hydrophobic material, it is suitable to disperse oil droplets into the aqueous phase. This relatively low productivity leads to the smaller scale application in industries (Nisisako *et al.,* 2003).

1.3.4 Microchannel emulsification

Kikuchi *et al.* (1992; 1994) proposed a microscope video system, in which a video recorder was attached on a microscope to observe microchannels (MCs) fabricated on a single crystal silicon substrate. This silicon chip was fabricated using semiconductor technique (Kikuchi *et al.,* 1992; 1994). Kawakatsu *et al.* developed a novel emulsification technique which is named microchannel emulsification (MCE) to prepare monodisperse emulsion using a single silicon-crystal MC array chip, and this process can be observed by microscope video system. There are two methods of MCE. The initially proposed one is called direct MCE in which the dispersed phase is passed through the MCs into continuous phase flows to form droplets. Direct MCE is able to produce monodisperse emulsion with the CV below 5%. The monodisperse emulsion produced by direct MCE is required very low energy input and no external force, which called "spontaneous droplet generation". The process of generated droplets by spontaneous transformation is very highly energy efficiency and mild (Sugiura *et al.,* 2001). One of advantages of direct MCE is the very mild process is just driven by interfacial tension when using some thermos-sensitive components, such as starch, and protein. The flow rate of continuous phase cannot influence the droplet size and uniformity (Kobayashi *et al.,* 2002). The droplet size will be not influenced by the flow rate of the dispersed phase below a critical value (Kobayashi *et al.,* 2001). There are two main types of direct MC array chips shown in Figure 1-5a and b. Grooved MC array chip (Figure 1-5a), consisted of parallel microgrooves and a terrace in the middle of chip.Straight-through MC array chip, consisted of arranged microthrough-

holes. We can clearly observe how the droplets generated in the MCs using grooved MC arrays. However, it is different to observe the phenomenon of the droplet generation that what happened to the water-oil interface inside the straight-through MCs. By contrast, the productivity of monodisperse emulsions produced by straight-through MC array chips is much higher than grooved MC array chips. The size uniformly sized droplets produced by direct MCE is around 1 μm to 500 μm using grooved MC array chip (Kobayashi *et al.,* 2007) or straight-through MC array chip (Kobayashi *et al.,* 2005, 2010). The direct MCE is able to prepare both W/O emulsions and O/W emulsion by using different kinds of surfactant (hydrophobic or hydrophilic) MC array chips (Kawakatsu *et al.,* 1996; 1997). Given an MC size, the productivity of straight-through MC array chip per unit area is 100 times larger than grooved MC array chip. The direct MCE using a straight-through MC array chip with an MC diameter of 10 μm enable producing monodisperse emulsion with the maximum dispersed phase flux around 0.06 $m^3/(m^2h)$ (Kobayashi *et al.,* 2003). Although the productivity of W/O emulsions by straight-through MC array chip is much higher than by grooved MC chip, the throughput is not available for large scale commercial production.

Premix MCE (Figure 1-6) is a novel and advanced technique based on direct MCE. This technique was proposed to increase productivity due to break-up the premix emulsion by passing through the grooved MC array chip (Kobayashi *et al.,* 2008). Premix MCE contains two steps: (1) to prepare premix emulsion using rotor-stator homogenization, (2) the droplets of premix emulsion flow through the MCs and polarize near the inlet of the MC array began to enter the inlet of terrace, and then break up into break up smaller ones

(Kobayashi *et al.*, 2008). Both straight-through MC array chip and grooved MC array chip can be used for premix MCE. W/O emulsion and O/W emulsion can be prepared by premix MCE by selection of MC array chips with hydrophobic or hydrophilic surfaces.

1.4 Hydrophobic surface treatment of membrane and microchannel array chips

1.4.1 Definition of surface hydrophobicity

The contact angle is defined by an angle formed by the solid surface and the tangent line to the upper surface at end point. The contact angle formation is driven by the surface or interfacial tensions between the solid and liquid surrounded by vapors. It is measured by the Young equation:

$$\gamma_s = \gamma_L \cdot \cos\theta\gamma_{SL} \quad (1\text{-}1)$$

where γ_L is the free energy of liquid surface, γ_s is the free energy of solid surface, γ_{SL} is the interfacial free energy of liquid/solid, θ is equilibrium contact angle. Young equation hypothesizes that the surface is totally flat, so it usually has a deviation in the resulting contact angle from the contact angle measured by Young equation, because the surface is always rough and impure in practical cases.

In general, we use contact angle on the surface of MC array chips to express the property of MC array chips. When the molecules containing in the liquid are forcefully attracted by the molecules containing in the solid, such as water on a totally hydrophilic solid, the liquid droplet will totally spread out on the surface of solid, the contact angle will be close to 0°. By contrast, when the molecules of a liquid are weakly attracted by the molecules containing in the solid, just part of liquid droplet will attach the surface of solid, the

contact angle will become higher. The water contact angle will be a range of 0° to 30° on highly hydrophilic surfaces of solid (Figure 1-7a). As shown in Figure 1-7b, the contact angle will be higher than 90° when the surfaces of solid are hydrophobic. The contact angle of solids may be higher than 120° when the solid surface made of lower energy (such as fluorinated) is highly hydrophobic. The water contact angle may even higher than 150° on the highly rough surface of some solids, since the presence of air pocket is under the liquid droplet. The materials surface mentioned above are called superhydrophobic. Some materials grammatically incomplete with smaller surface free energy theγ_s is small. The water drop will form a small contact angle on the surface of these materials. In contrast, materials such as glasses and metals are usually with a large surface free energy, the water drop will form a large contact angle on the surface. In some cases, gas is used to measure the contact angle of materials to replace liquid that the angles of 180° (mentioned above) minus will be replaced. The liquid water angle on materials is very sensitive to reflect the different results, even the surface of materials are contaminated by a small amount of orders. As a result, it is useful to use contact angle to express the hydrophobicity of materials surface.

In general, we use the $\theta/2$ method called a half angle method to calculate the contact angle. As shown in Figure 1-8, the contact angle is determined by the method from the angleθ_1 between the the line passing the apex of droplet and the base line on the surface of materials. $2\theta_1$ is equal to θ, which is based on the assumption the droplet profile forms a segment of arc. This method is based on the formed droplet is a partial sphere. The measurement result may be inaccurate when the formed droplet is not a sphere.

1.4.2 Surface property of chip materials

Single-crystal MC array chips are usually used to prepare monodispers emulsion in MCE. In order to produce monodisperse W/O emulsions, the surfaces of silicon MC array chip must be sufficiently hydrophobic. Polymeric MC array chips are intrinsically hydrophobic to produce W/O emulsions. Prior to the first usage, the MC array chips are treated by the plasma oxidation to clean their surfaces, and then the MC array chips are soaked in the hydrophobic surfactants to keep the surface hydrophobic. Moreover, the osmotic of dispersed phase has to be high enough to suppress the water molecules through the water-oil interface. The surface of metals such as stainless steel is always rough, that the hydrophobicity of stainless-steel is primarily better than surface-oxidized silicon. Stainless-steel chips also have some advantages compared with silicon array chip. For a practical point of view, the silicon is weak to alkali cleaning which is usually used in industry. Stain steel has better chemical-proof surface and mechanical strength against alkali cleaning. The better mechanical strength of stainless-steel ensure it appropriate for repeated use and handing in the food industries (Tong *et al.,* 2001). The MC array chips cannot keep long term of hydrophobicity more than one year after hydrophobic treatment, the contact angle to the materials surface will decrease and lost their hrdrophobicity. To our knowledge, there is no study to produce W/O monodisperse emulsions by direct MCE using stainless-steel chip. There is also a room to prepare monodisperse W/O emulsions using premix MCE to increase the productivity.

1.4.3 Hydrophobic treatment of membrane and microchannel chips

W/O emulsification is an important process in cosmetic, food, chemical, and pharmaceutical industries. The high quality and stability of industrial products require the resulting emulsion is of monodispersity. In order to prepare monodisperse W/O emulsions, the emulsification devices (e.g. membrane, MC array chip) must be hydrophobic (Kawakatsu *et al.,* 2002).

There is less number of researches that focus on preparing W/O emulsions by membrane emulsification, since it is more difficult to produce W/O emulsions compared with O/W emulsions. This fact can be explained by that the aqueous droplets are difficult to keep stable in oil phase with a low dielectric constant are no electrical double-layer repulsion forces (Kandori *et al.,* 1991). The preparation of W/O emulsion by membrane emulsification devices in previous studies was mostly performed using porous glass membranes (Kandori *et al.,* 1991; Nakashima *et al.,* 1992). In general, there are two approaches to make the microporous glass membranes surface hydrophobic: (i) the porous glass membranes are fabricated with silane coupler reagents, (ii) the membranes are immersed in oil for a certain period of time (Katoh *et al.,* 1993; 1997). The production of monodisperse aqueous droplets by membrane emulsification has been widely used in the productionW/O emulsion-based products, such as microcapsules, low-fat spreads, and hydrogel microparticles. However, membrane emulsification using porous glass membranes is just used in W/O emulsion containing sub-micron or micron scale with the aqueous droplest size on 10 μm, since the pore size distribution of membrane is in this range.

Tan *et al*. (2006) reported to prepare monodisperse lipid vesicles prepared by microfluidic emulsification. In the device, the microfluidic channel surface treatment method is based on microchannel emulsification (introduce in the following chapter). As the high monodispersed droplet can be prepared, microfluidic emulsification is suitable to W/O emulsion food products, e.g. butter and margarine, and also some ice cream, chocolate which containing air-in-oil bubbles.

Monodispersed W/O emulsions can also be produced by MCE using hydrophobically surface-treated MC array chip (Kawakatsu *et al.*, 2001; Kobayashi *et al.*, 2008; 2009). This technique has similar droplet generation mechanism with that in the membrane emulsification. Therefore, hydrophobic MC array chips are necessary to produce monodisperse W/O emulsions. In this technique, appropriate hydrophobic surfactants are required for the successful production of W/O emulsions,since continuous phase can preferably wet onto the MC surface rather than dispersed phase (Sugiura *et al*., 2001). Silicon ME array chips are normally used for preparing monodisperse W/O emulsions by MCE. Polymeric MC array chips with intrinsically hydrophobic surface are also used to prepare W/O emulsions (Liu *et al.*, 2004; Kobayashi *et al.*, 2008; 2009). Moreover, the enough high osmotic pressure of the dispersed phase is an important factor to suppress the water molecules migration through the water-oil interface (Kobayashi *et al.*, 2009). Alkane oils which have low viscosity are normally applied as continuous-phase medium in MCE. Kobayashi *et al*. (2009) reported that triglyceride oils are also able to use as continuous-phase medium.

1.5 Objectives and constitution of the thesis

To our knowledge, there is no research focusing on preparation of monodisperse W/O emulsionsby direct MCE using a hydrophobic stainless-steel MC array chip.

The objectives of this thesis are to clarify the operation and suitable storage condition of hydrophobicity of MC array chips, and to generate monodisperse W/O emulsions using MCEs (direct MCE by stainless-steel MC array chip and premix MCE by silicon MC array chip). This thesis also investigates the break-up process of aqueous dropletsduring premix MCE. We also focused on the droplet size and its distribution, the flow rate of disperse phase, productivities of resulting W/O emulsions, and the capillary number to analyze the characteristics of the resultant W/O emulsions.

Figure 1-9 shows the structure of the thesis. This thesis is started by the general introduction, including the emulsion overview, emulsification devices and the hydrophobicity of MC array chips (Chapter 1).

The thesis is mainly composed of three parts. In chapter 2, we focus on the effect of surface treatment methods and storage conditions, such as storage time and temperature, on the hydrophobicity of MC array chips, which is important for producing W/O emulsions. The second part introduces the generation of W/O monodisperse emulsions by direct MCE using hydrophobic stainless-steel MC array chip (Chapter 3). In this chapter, we also analyzed the characteristics of resultant W/O emulsions. The third part is focus on the preparation characteristics of monodisperse W/O emulsions by premix MCE using hydrophobic stainless-steel MC array chips which is able to increase the productivity (Chapter 4).

Finally, the thesis ends with the conclusions and perspectives in chapter 5.

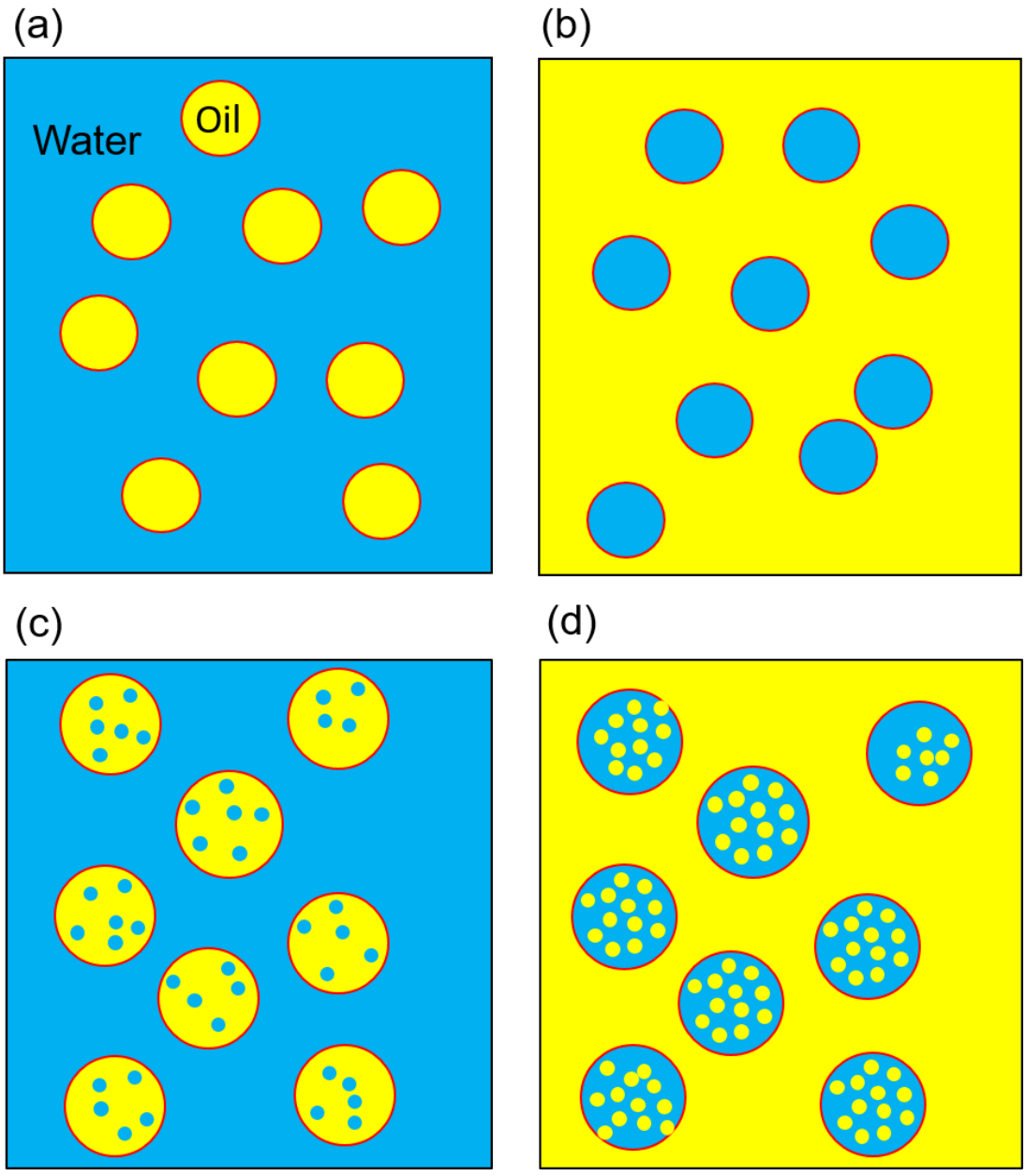

Figure 1-1. Schematics drawing of emulsion types. (a) oil-in-water (O/W) emulsion. (b) water-in-oil (W/O) emulsion. (c) water-in-oil-in-water (W/O/W) emulsion. (d) oil-in-water-in-oil (O/W/O) emulsion.

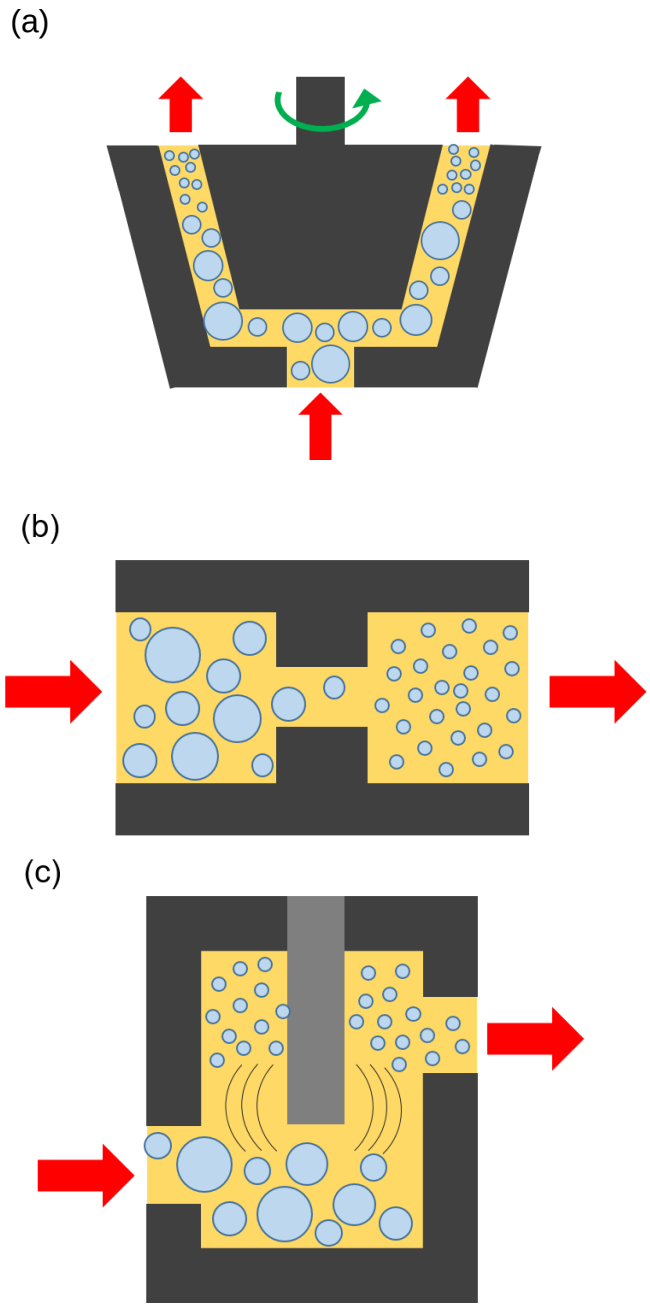

Figure 1-2. Schematic representation of conventional emulsification methods. (a) Rotor-stator homogenizer. (b) High pressure homogenizer. (c) Ultrasound homogenizer.

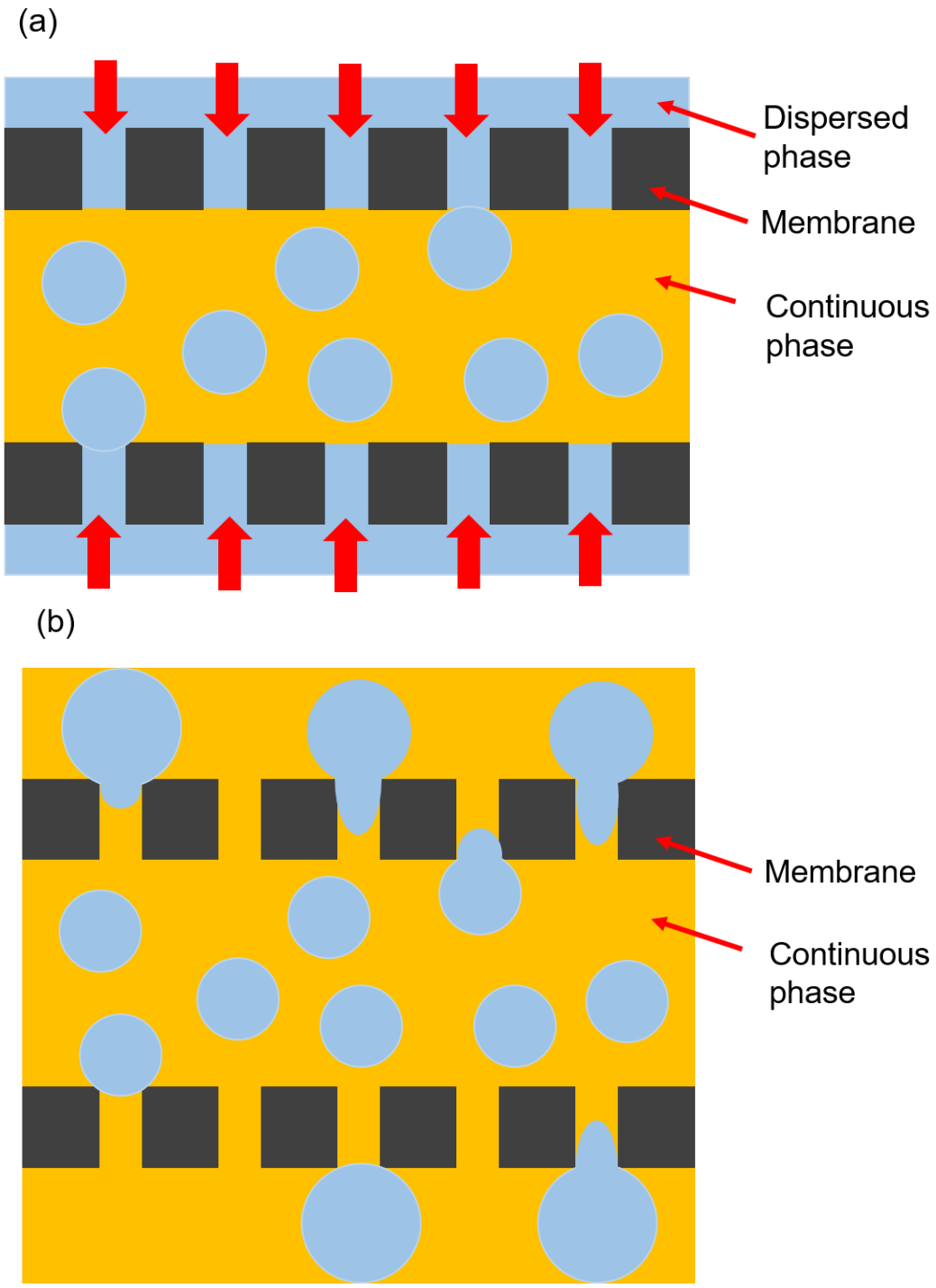

Figure 1-3. Schematic diagram of membrane emulsification process. (a) Direct membrane emulsification process. (b) Premix membrane emulsification process.

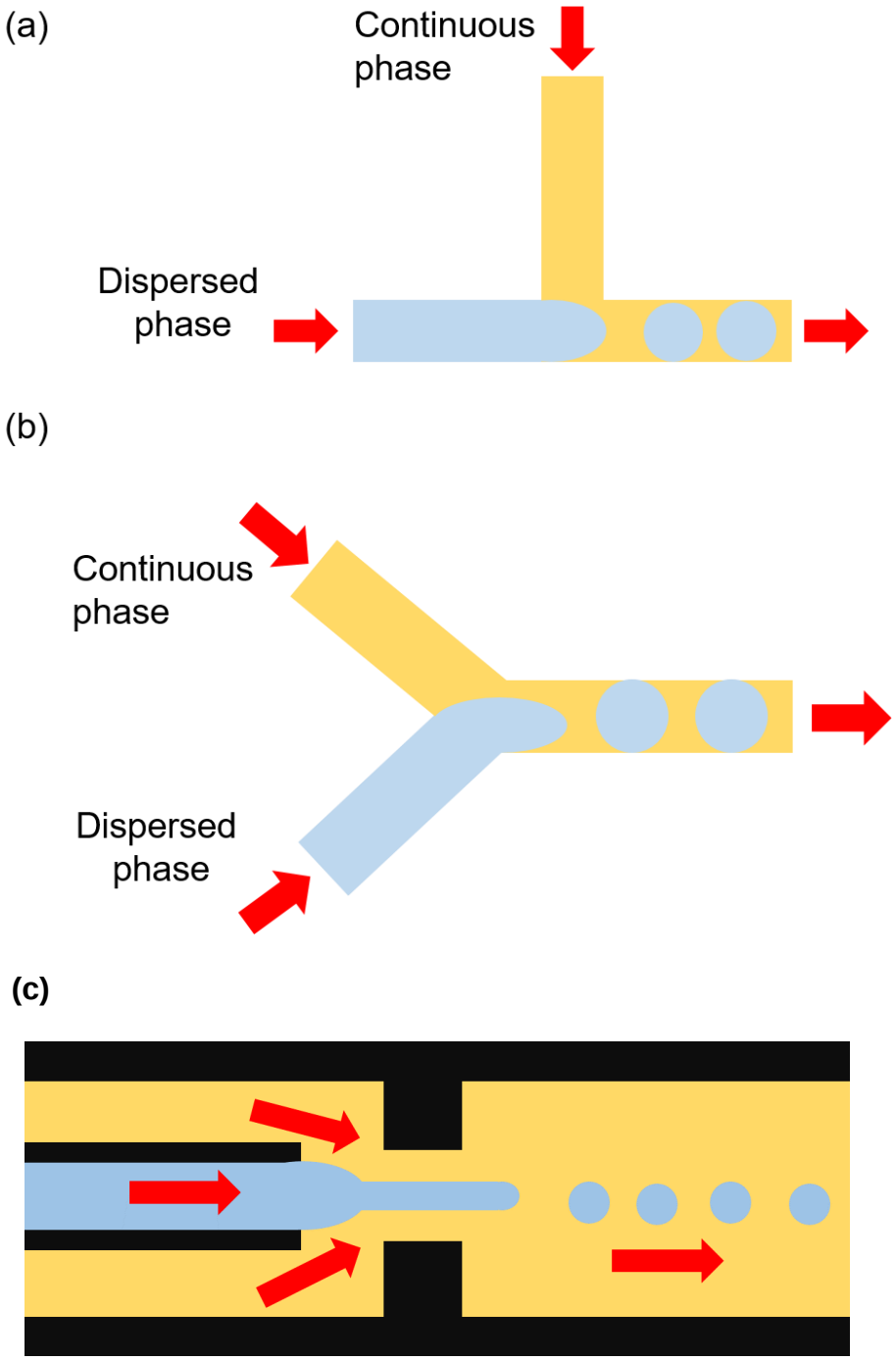

Figure 1-4. Schematic diagram of the microfluidic emulsification process. (a) T-junction. (b) Y-junction. (c) Microfluidic flow focusing device.

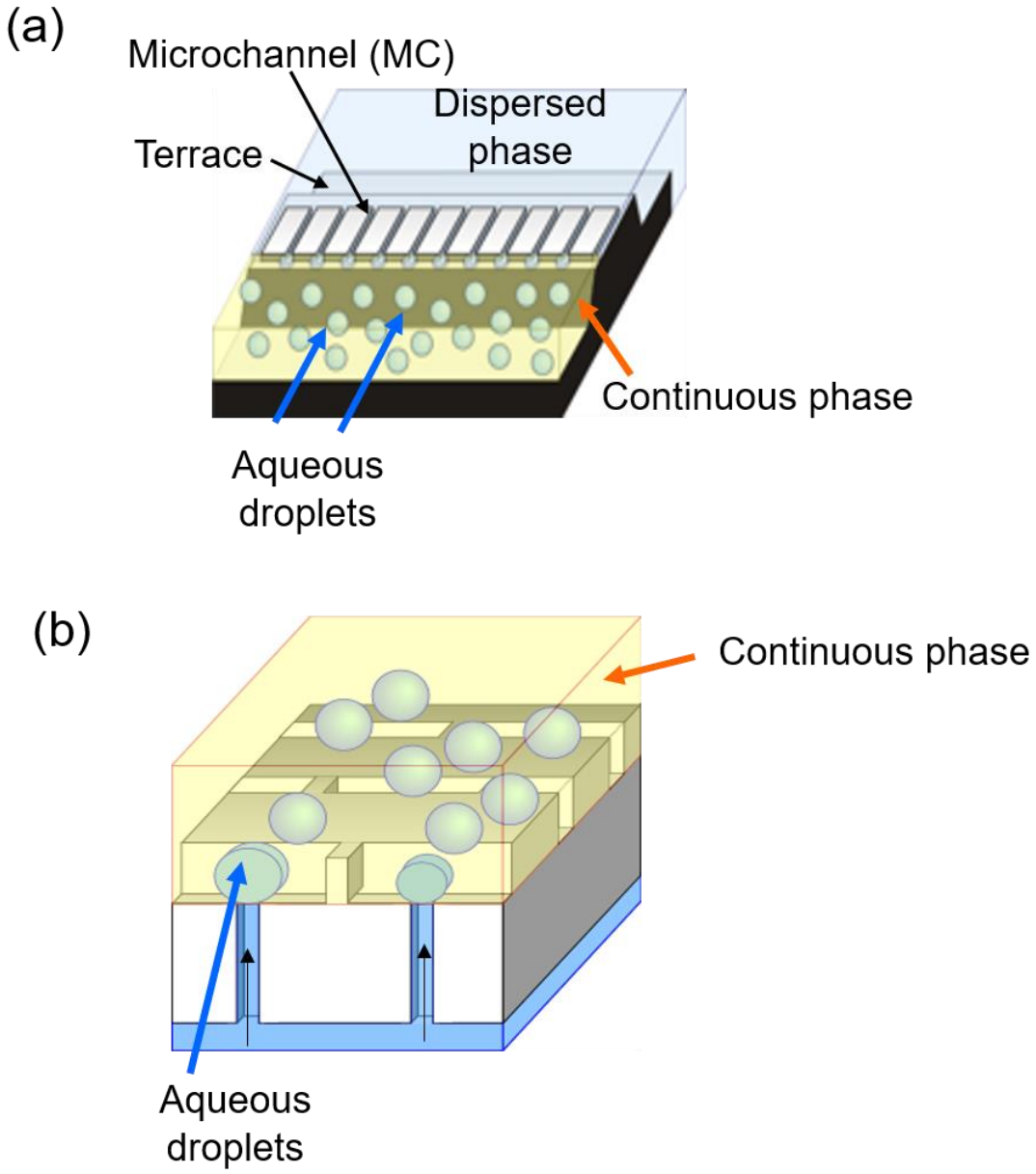

Figure 1-5. Schematic drawings of droplet generation by direct MCE. (a) Part of grooved MC array. (b) Part of straight-through MC array.

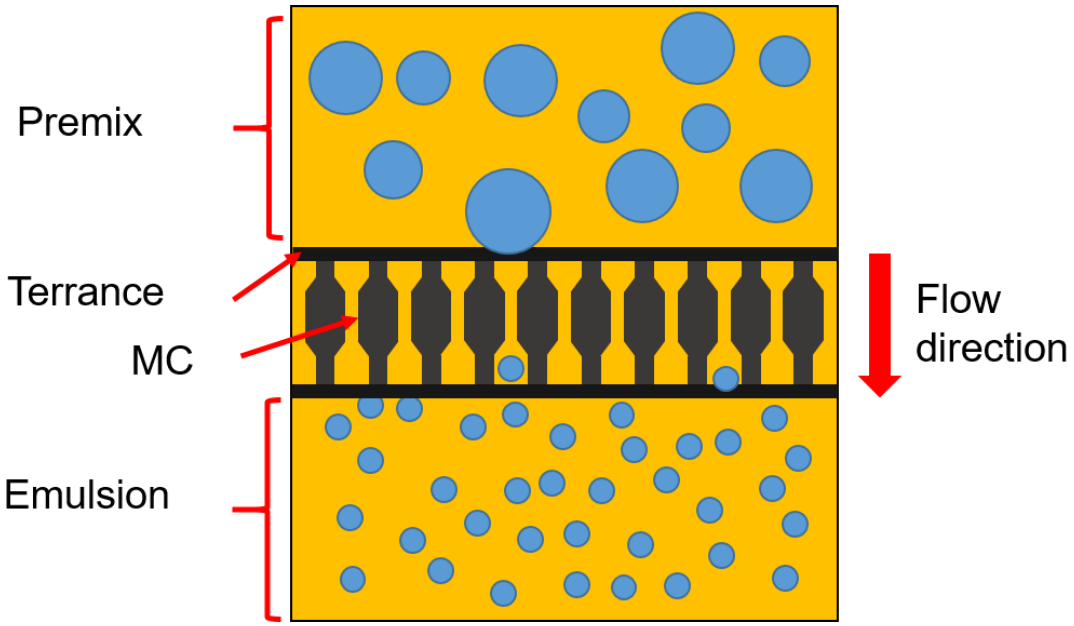

Figure 1-6. Schematic drawing of droplet generation by premix MCE.

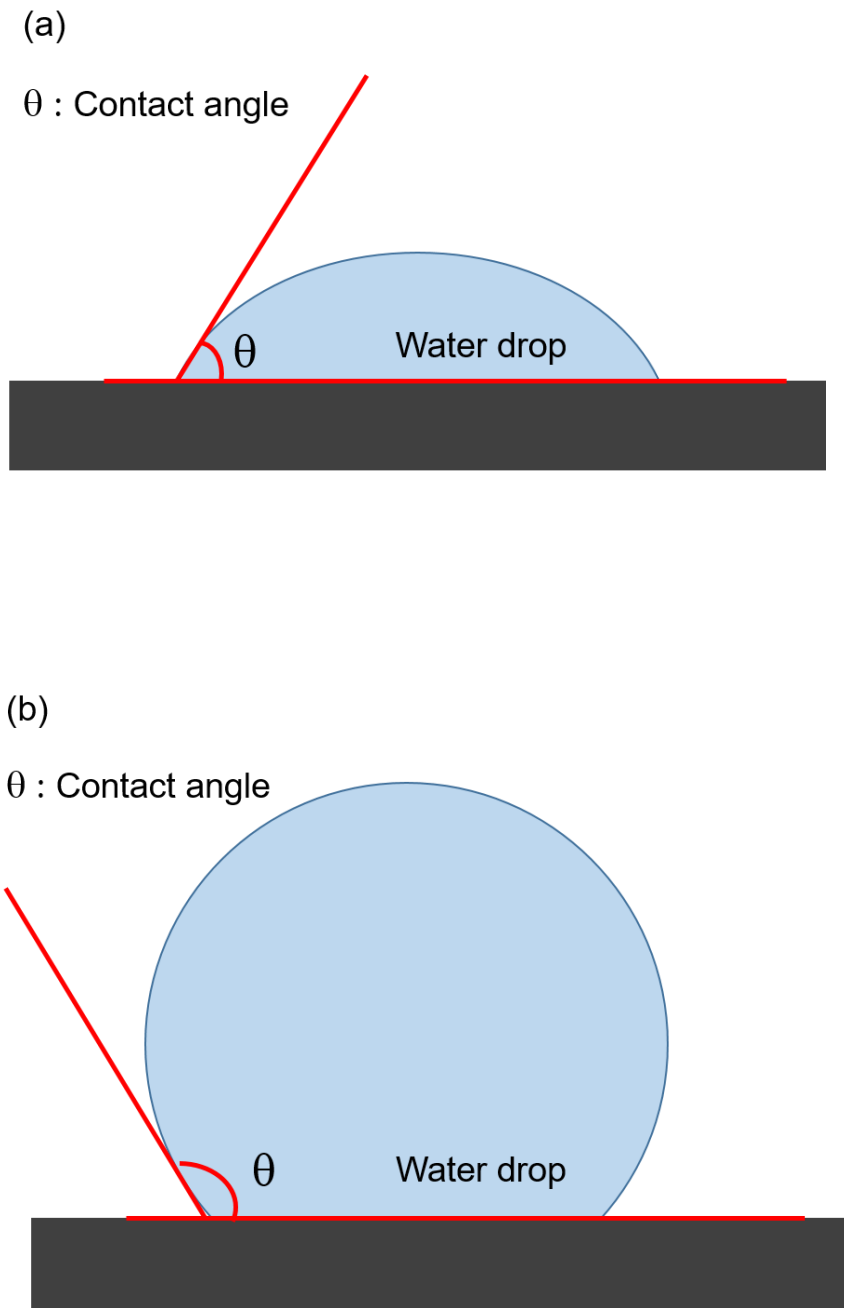

Figure 1-7. Schematic drawing of typical contact angle of the solid surface. (a) Hydrophilic surface of solid with a contact angle lower than 90°. (b) Hydrophobic surface of solid with a contact angle larger than 90°.

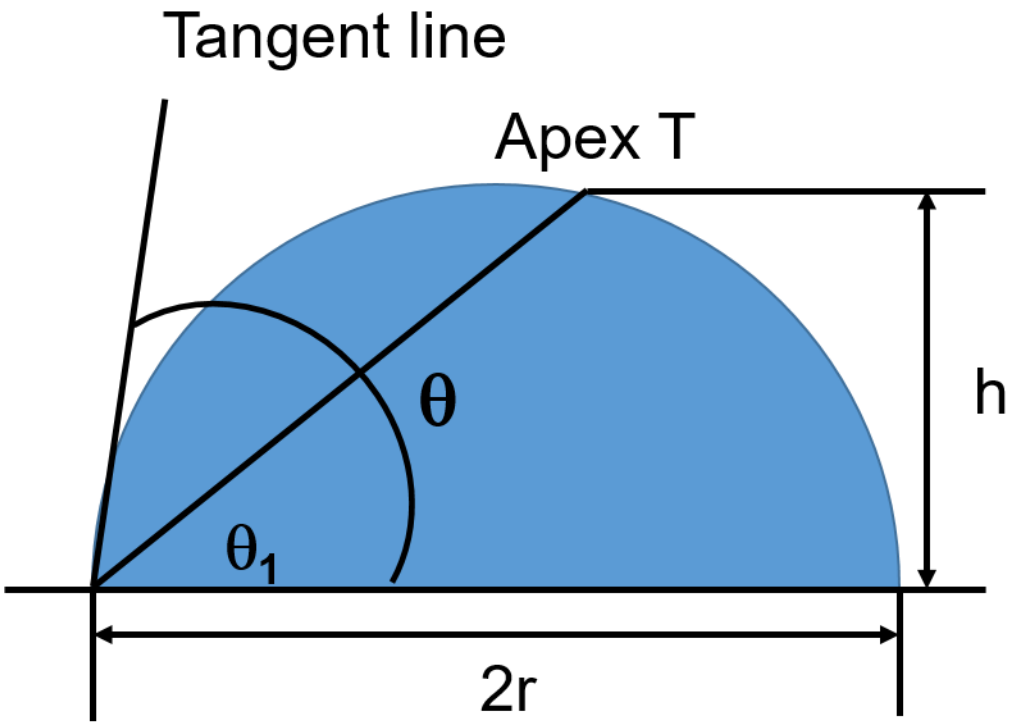

Figure 1-8. Schematic drawing of θ/2 method.

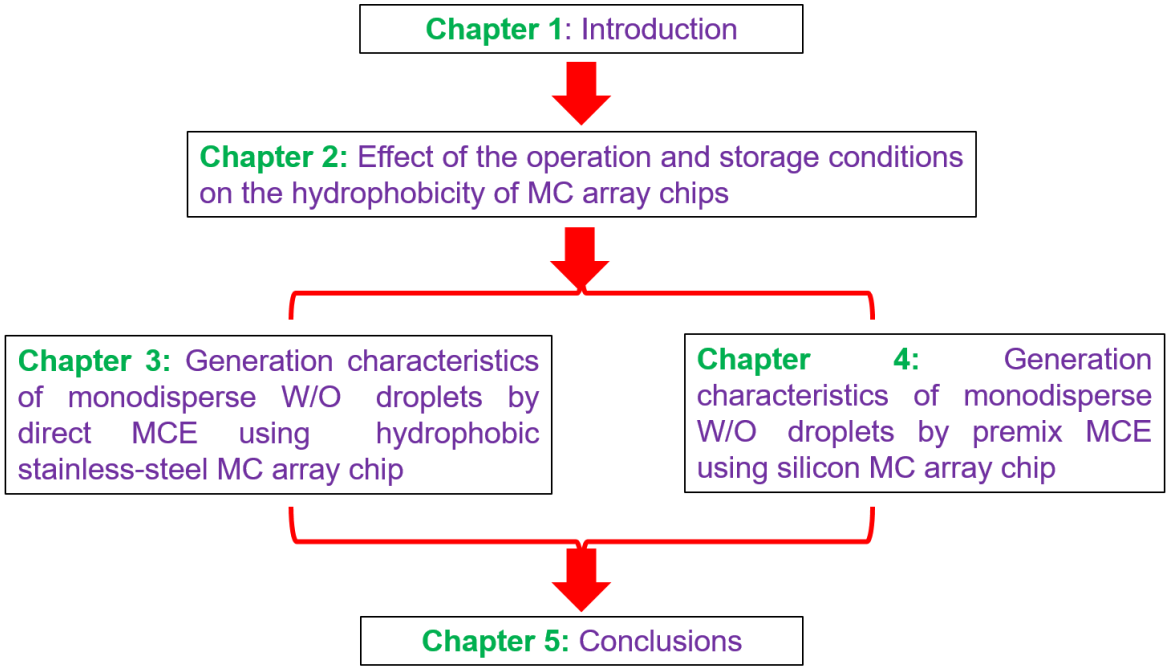

Figure 1-9. The general structure of thesis.

Chapter 3
Generation characteristics of monodisperse water-in-oil emulsions by direct microchannel emulsification using hydrophobic stainless-steel microchannel array chip

3.1 Introduction

Water-in-oil (W/O) emulsions play an important role in various products, such as foods, pharmaceutics, medicines, chemicals and cosmetics. They are also applied to prepare water-in-oil-in-water (W/O/W) emulsions as a dispersed phase to encapsulate bioactive substances that are hydrophilic (Zanattaa *et al.,* 2017; Khalid *et al.,* 2016). W/O emulsions consisting of large aqueous droplets with sizes of 50 μm to 1000 μm are commonly prepared as templates to produce microcapsules and microparticles by using polymerization, gelation, solidification and other secondary processes. Uniform-sized large droplets used as templates for carrying bioactive substances make it easier to control the release of biological materials in microcapsules and microparticles (Khalid*et al.,* 2016).

Various traditional emulsification techniques are used to produce W/O emulsions, e.g., high-pressure homogenizer, colloid mills, and rotor-stator systems. The size range of the obtained droplets is normally broad from 0.1 μm to 100 μm. The resultant emulsions are usually polydisperse with a broad droplet size distribution (typically >20%, having lower stability (e.g., coalescence and sedimentation) that may affect the physical quality of products (Khalid *et al.*, 2015).

In the past two decades, some advanced technologies capable of producing monodisperse W/O emulsion have been rapidly developed. Membrane emulsification (ME) is used for preparing relatively monodisperse W/O emulsions with the smallest coefficient of variation (CV) of about 10% (Nakashima *et al.,* 2000). ME is normally used to prepare droplets smaller than 100 μm in size by forcing a disperse phase through membrane pores into a continuous-phase area. This technique requires low-energy input, as droplet

generation occurs at low mechanical stress. Single-crystal silicon, Shirasu Porous Glass (SPG), and stainless steel are commonly used as the membrane materials. Cleaning after ME is time-consuming and inconvenient due to the 3D interconnected membrane pores (Nakashima *et al.,* 1991; Vladisavljević and Williams 2006). Microfluidic emulsification (MFE) which has different geometries such as Y-junction, T-junction and cross-junction can also produce highly monodisperse W/O emulsions with the smallest CV of about 3%. This technique can produce aqueous large droplets with sizes exceeding 100 μm. The materials commonly used for microfluidic emulsification are poly (dimethylsiloxane) (PDMS) and glass (Vladisavljević *et al.*, 2012).

Microchannel emulsification (MCE) is a promising technique that enables preparing monodisperse W/O emulsions by injecting a disperse phase into a continuous phase through microchannel (MC) arrays (Kawakatsu *et al.,* 1997; Kobayashi *et al.,* 2009). The droplets generated by direct MCE, which exploits the interfacial tension with low-energy input, are uniformly sized with a CV below 5% (Sugiura *et al.,* 2001). It is promising that MCE can produce uniform and large aqueous droplets with sizes exceeding 100 μm when using MC arrays, each consisting of large MC and terraces.

A hydrophobic silicon MC array chip is usually required for direct MCE to prepare W/O emulsions (Kobayashi *et al.,* 2009). However, a silicon-based MC array chip has some disadvantages compared with a stainless-steel MC array chip. For instance, a silicon chip is fragile and prone to break easily, and is sensitive to alkali cleaning. In contrast, a stainless-steel chip has mechanical strength and chemical stability against alkali cleaning frequently used in food industries (Kobayashi *et al.,* 2012).

In the previous study, there was no research focusing on W/O emulsions by direct MCE using a hydrophobically surface-treated stainless-steel MC array chip. Therefore, the objective of this study was to generate aqueous droplets using hydrophobic stainless-steel arrays. The MC dimensions play an important role in the uniform-sized droplets generation. We also analyzed the characteristics of W/O emulsions in terms of the droplet size, its distribution, and the flow rate of disperse phase on the droplet generation.

3.2 Materials and methods

3.2.1 Emulsification setup

Figure 3-1a depicts the stainless-steel MC array chip used for MCE in this study. The chip has a flow channel with two through-holes that will be filled with continuous phase and two MC arrays beside the flow channel. The positions of the terrace and parallel channels on each MC array are indicated in Figure 3-1b. Table 3-1 presents the dimensions of the stainless-steel MC array chip used for MCE in this study. The stainless-steel MC arraychips have different MC depths of 30 μm (SSMC-30) and 100 μm (SSMC-100). The MC widths are 35 μm (SSMC-30) and 100 μm (SSMC-100), respectively. According to previous study (Sugiura *et al.,* 2002), the ratio of MC width/depth is close to 1:1 used in this study means that droplets can be more stably generated.

3.2.2 Preparation of solutions

The continuous phase was pre-treated decane (Wako Pure Chemical Ind., Osaka, Japan) containing 5 wt% tetraglycerin monolaurate condensed ricinoleic acid ester (CR-310),

purchased from Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan), used as surfactant. The dispersed phase was Milli-Q water solution consisted of 5 wt% polyethylene glycol (PEG,molecular weight20,000) and 5 wt% NaCl (Wako Pure Chemical Ind.). The density of two phases in this study were 738 kg/m^3 (continuous phase) and 1042 kg /m^3 (disperse phase), respectively. The viscosities of continuous phase and disperse phases were 0.96 mPa/s and 1.02 mPa/s, respectively. The interfacial tension of water-in oil systems used in this study was 3.6 mN/ m.

3.2.3 Microchannel emulsification

Before each MCE experiment, the stainless-steel MC array chip was soaked in hexamethyldisilazane (LS-7150, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) for 24 h to render the chip surface hydrophobic. Afterwards, the MC array chip was cleaned using Milli-Q water for 20 min by an ultrasonic bath (VS100III, As One Co., Osaka, Japan). The MCE setup used in this study consists of a module whose compartments were previously filled with continuous phase and two syringe pumps (Model 11, Harvard Apparatus Inc., Holliston, USA) used to inject continuous and dispersed phases into the module, which was equipped with vitreous syringes. The dispersed phase was gradually fed into the MC array chip soaking in the module via a plastic tube to go through the parallel channels to generate aqueous droplets. The MCE procedure was monitored through a microscope video system (Kobayashi *et al.*, 2012).

3.2.4 Measurements and analyses

The water contact angle on the surface of a stainless-steel MC array chip was measured with a fully automatic interfacial tensionmeter (PD-W, Kyowa Interface Sciences Co., Ltd., Saitama, Japan). For each stainless-steel MC arraychip, we dispensed 10 water drops with a volume of 0.5 mL onto the chip surface by an automatic syringe.

The aqueous droplet size and its distribution by MCE were determined as follows. The average droplet diameter (d_{av}) was defined by:

$$d_{av} = \sum_{i=1}^{n} d_i / n \quad (3\text{-}1)$$

where d_i is the diameter of the *i*-th droplet measured by WinRoof software (Mitani Co., Ltd., Fukui, Japan) and *n* is the number of droplets measured (n = 100). The droplet size distribution (CV) is defined as:

$$CV = (\sigma / d_{av}) \times 100 \quad (3\text{-}2)$$

where σ is the standard deviation.

3.3 Results and discussion

3.3.1 Surface properties of hydrophobic stainless-steel microchannel array chips

In order to successfully prepare W/O emulsion, the chip surface must be hydrophobic. The water contact angle of the stainless-steel MC chip surface after hydrophobic treatment was measured in two ways: water phase in air and water phase in oil phase. The water contact angle is a critical factor in determining the surface properties. If the contact angle is higher than 90°, the surface of the material is hydrophobic. In contrast, if the contact angle is lower than 90°, the surface of material is hydrophilic (Butron Fujiu *et al.*, 2011). After surface treatment, the contact angle was 95.1° for water phase in air and 157.3° for water

phase in oil phase (Figure 3-2). Both of these water contact angles exceed than 90°, demonstrating that surfaces of the stainless-steel MC array chips used in this study were totally hydrophobic. The resultant contact angles were a little less than those obtained for a hydrophobic silicon array chip in the previous research which used the same method for hydrophobic treatment (Butron Fujiu *et al.*, 2012). This may because the reagent used for hydrophobic treatment, a silane coupler reagent, made coupling with the surface of the silicon chip easier. The hydrophobicity of a stainless-steel MC array chip can be maintained for almost one month, which was close to the value for a silicon MC array chip (over one month). Sufficient hydrophobicity of the stainless-steel MC array chip surface could assure the successful generation of aqueous droplets wetted by continuous phase rather than wetted by dispersed phase.

3.3.2 Droplet generation characteristics

Figures 3-3a and 3-3b present the typical optical micrographs of the successful generation of aqueous droplets via hydrophobic stainless-steel MC arrays (SSMC-30 and SSMC-100). In both systems, the disperse phase passes through the MCs to generate uniform-sized droplets. The flow rate of the continuous phase was 9 mL/h while that of the disperse phase was 0.5 mL/h in Figures 3-3a and 3-3b. The d_{av} and CV of the resultant aqueous droplets were108.2 μm and 3.1% for SSMC-30 and 298.5 μm and 4.5% for SSMC-100. Figures 3-3c and 3-3d indicate that the droplet sizes had very narrow distributions. These results strongly demonstrate that both of the stainless-steel MC array chips were able to generate highly uniform-sized aqueous droplets. The ratio of d_{av}/h_{MC} was 3.61 for SSMC-

30 while the radio of d_{av}/h_{MC} was 2.96 for the SSMC-100 array chip. Both d_{av} values were 3-3.5 times the MC depth, being reasonable according to previous research focusing on the hydrophobicity of silicon MC array chips (Butron Fujiu *et al.*, 2012).

3.3.3 Effect of disperse phase flow rate during W/O emulsion preparation

Figure 3-4a shows the effect of the disperse phase flow rate (Q_d) on droplet size in the W/O emulsions for the stainless-steel MC array (SSMC-30 and SSMC-100). For the SSMC-30 chip, the d_{av} of the generated aqueous droplets increased from 109 μm to 187 μm as Q_d was gently increased from 0.2 mL/h to 1.1 mL/h. For the SSMC-100 chip, their d_{av} increased from 297 μm to 365 μm as Q_d was gently increased from 0.3 mL/h to 2.0 mL/h. The critical Q_d values for the SSMC-30 and SSMC-100 chips were 0.6 mL/h and 1.3 mL/h, respectively. Below the critical Q_d, the stainless-steel MC arrays could produce monodisperse W/O emulsions with uniform-sized droplets. In contrast, they became non-uniform and larger with increasing Q_d above the critical value. There was a constant interval for droplet generation from each MC. Moreover, the water-oil interface stably moved in the MC filled with continuous phase and on the terrace to generate uniform-sized droplets. Above the critical Q_d, there was not sufficient time for the water-oil interface to expand and pinch-off on the terrace, resulting in larger and non-uniform droplets. The critical Q_d for SSMC-100 was about twice that for SSMC-30. The different critical Q_d values in these two systems were determined by the different MC dimensions, since the same continuous and disperse phases were used. The trends shown in Figure 3-4 were

probably appropriate, because similar trends have been obtained during preparation of W/O emulsion using silicon MC array chips by MCE (Butron Fujiu *et al.,* 2012).

The velocity of the disperse phase in an MC (U_d), which is an important element for analyzing the flow state of the disperse phase during emulsification, which is given by:

$$U_d = [(\pi/6)d_{drop}^3 f_{MC}]/A_{MC} \quad (3\text{-}3)$$

where A_{MC} is the MC cross-sectional area and d_{drop} is the droplet diameter. f_{MC} is the droplet generation frequency at an MC. f_{MC} is estimated by:

$$f_{MC} = 1/t \quad (3\text{-}4)$$

where t is the droplet generation time, which was calculated as the average time of ten droplets. In Figure 3-4b, f_{MC} obviously increased with increasing U_d below its value of 16.2 mm/s for the SSMC-30 and 23.5 mm/s for the SSMC-100. In contrast, f_{MC} smoothly decreased with increasing U_d above its critical values for both SSMC-30 and SSMC-100. The main reason for the difference in critical U_d values in Fig. 5 is considered to be the different MC depth and width. The preceding results illustrates that uniform droplets can be generated by the hydrophobic stainless-steel MC array chips at a milliliter per hour scale. The maximum productivity of monodisperse W/O emulsion of SSMC-100 exceeded that of SSMC-30 by about 2 times.

3.3.4 Analysis of capillary number during water-in-oil emulsion preparation

The capillary number of the disperse phase that flows in an MC is an important parameter affecting droplet generation via an MC array (Sugiura *et al.,* 2002). The capillary number of dispersed phase (Ca_d) is defined by:

$$Ca_d = \eta_d U d / \gamma \quad (3\text{-}5)$$

where η_d is the viscosity of the dispersed-phase and γ is the interfacial tension between the continuous and disperse phases. In Figure 3-5, the d_{av} obviously increased at the critical Ca_d of 0.016 for SSMC-30 and 0.024 for SSMC-100. Below the critical Ca_d value, d_{av} isalmost constant, indicating that the aqueous droplets are uniform-sized. In contrast, d_{av} clearly became larger with increasing Ca_d above the critical value for both SSMC-30 and SSMC-100. Thus, the stainless-steel systems of SSMC-30 and SSMC-100 were capable of generating uniform-sized droplets below the critical Ca_d. The critical Ca_d value for SSMC-100 is about 1.5 times that for SSMC-30. This difference is smaller than the difference in Q_d between SSMC-30 and SSMC-100, which is attributed to the difference in the MC cross-sectional size.

3.4. Conclusions

The results in this study demonstrate that hydrophobic stainless-steel MC array chips can prepare monodisperse W/O emulsions with uniform droplet size, with CV below 5% for each chip. The hydrophobicity of stainless-steel MC array chips was high enough to generate aqueous droplets, which was proved by the contact angle results. The aqueous droplets obtained using the hydrophobic stainless-steel MC arrays were larger than 100 μm and were mainly affected by the MC depth. Below the critical Ca_d, uniform-sized aqueous droplets can be generated stably. Their maximum productivity was observed around the critical Ca_d droplets prepared in this study have potential food and pharmaceutical applications, such as beads, capsules, living cells and lactobacilli.

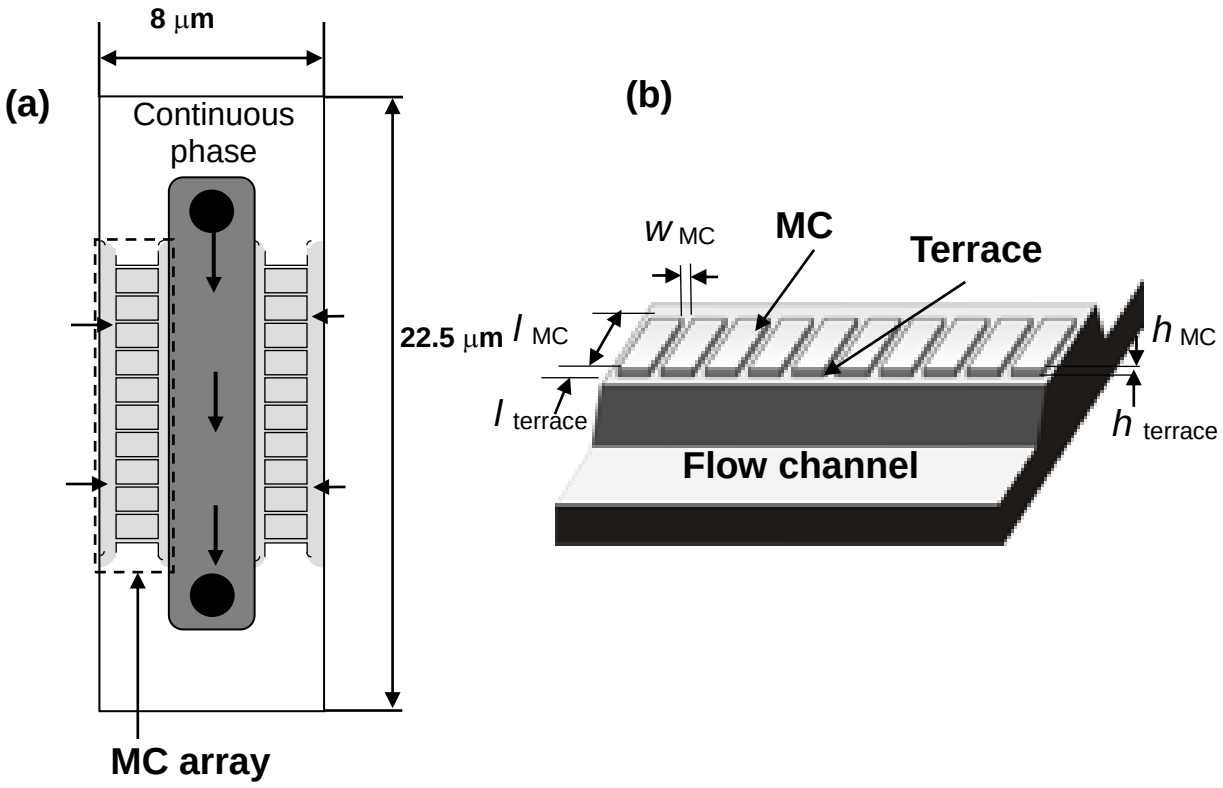

Figure 3-1. (a) Schematic top view of a stainless-steel MC array chip. (b) 3D drawing of part of an MC array.

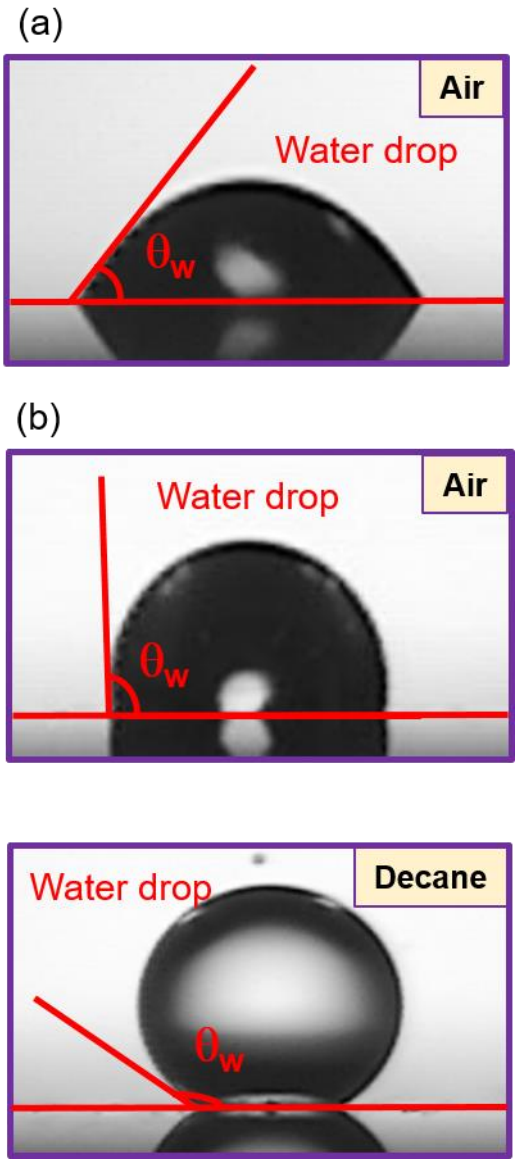

Figure 3-2. (a) Contact angle of a water drop to a stainless-steel MC array chip in air before hydrophobic treatment. (b) Contact angle of a water drop to stainless-steel MC array chip in air or decane after hydrophobic treatment.

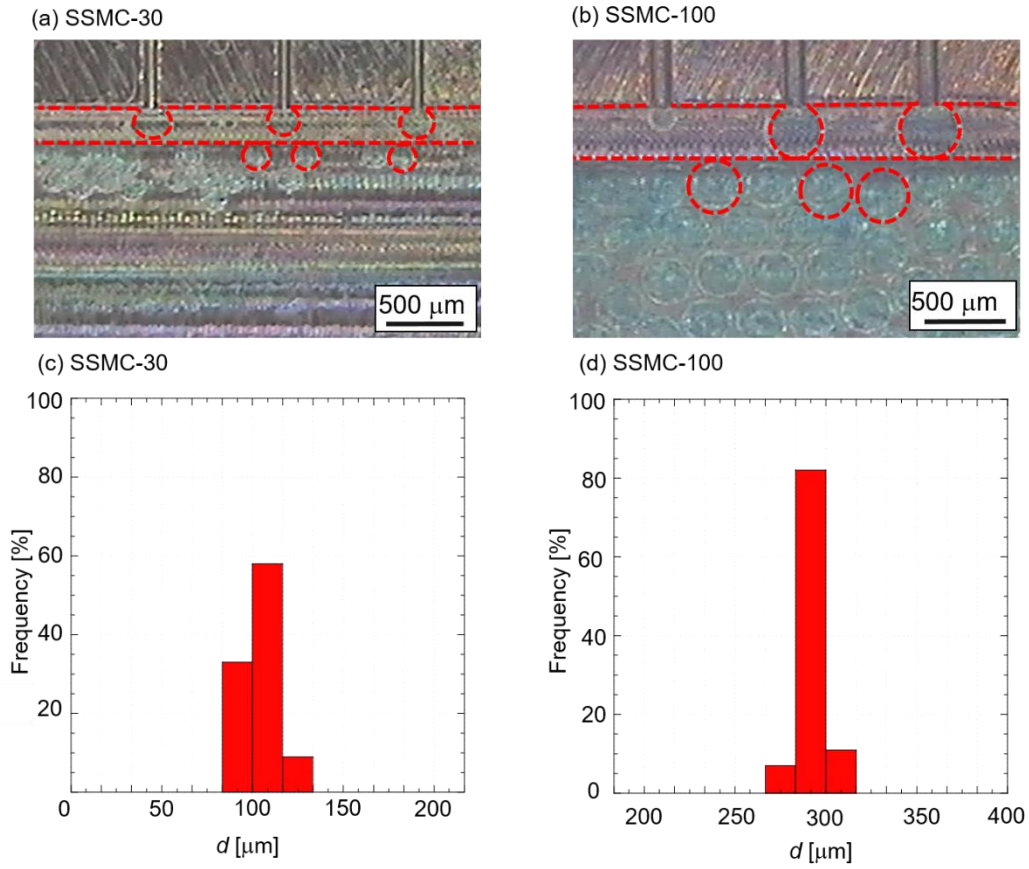

Figure 3-3. (a,b) Optical micrographs of aqueous droplet generation using hydrophobic stainless-steel MC arrays. (c,d) Size distributions of the aqueous droplets generated using hydrophobic stainless-steel MC arrays.

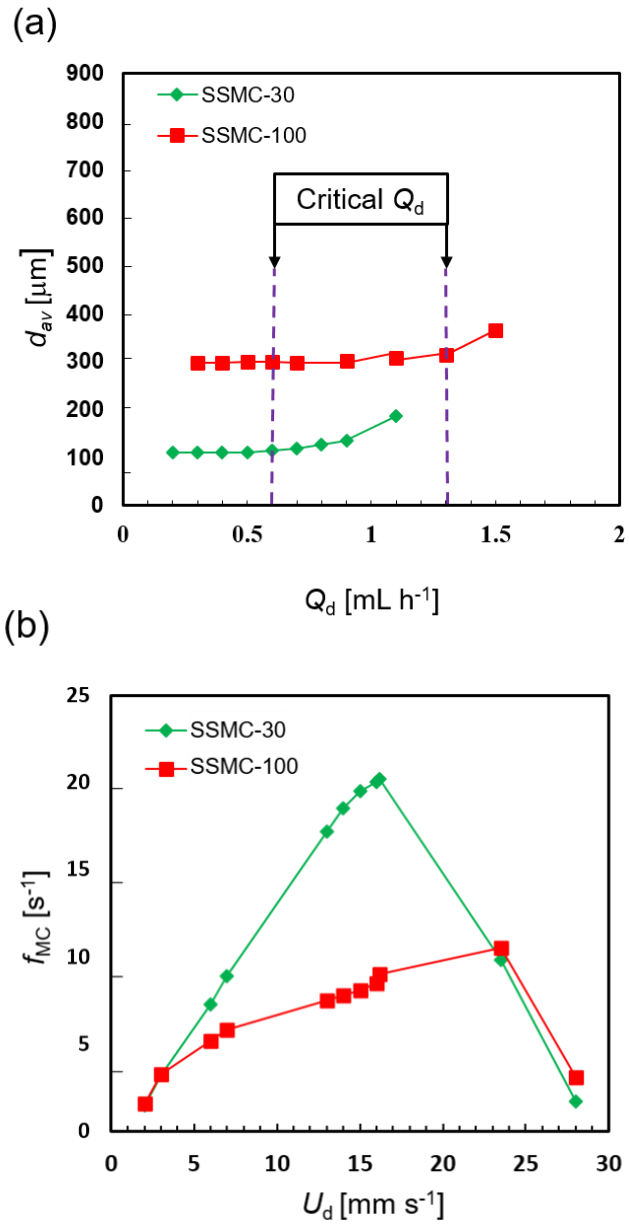

Figure 3-4. (a) Effect of the dispersed phase flow rate (Q_d) on average diameter (d_{av}) of the aqueous droplets generated using hydrophobic stainless-steel MC array chips. (b) Effect of the dispersed-phase velocity in an MC (U_d)on the droplet generation frequency (f_{MC}).

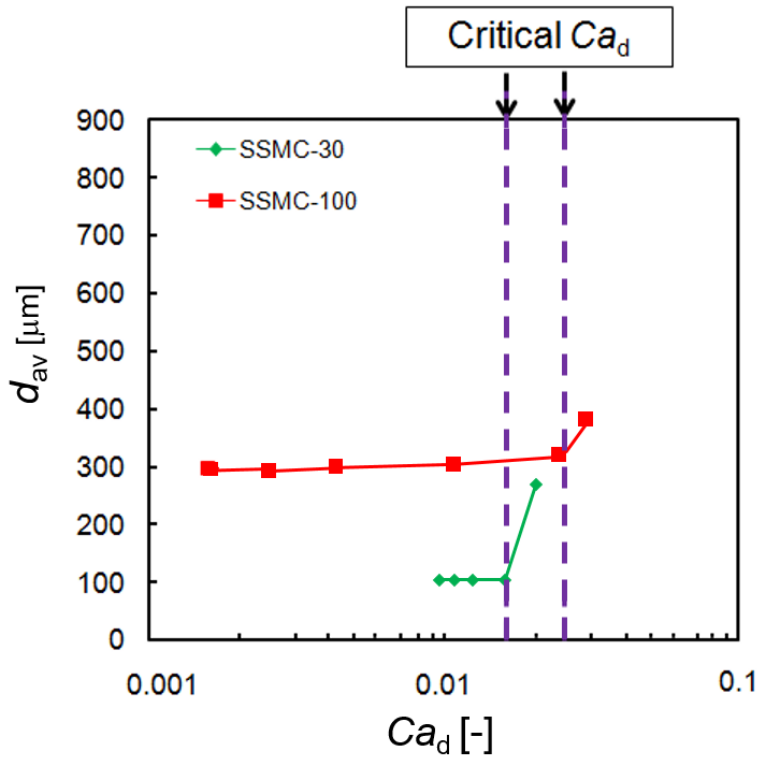

Figure 3-5. Effect of the capillary number of the dispersed phase that flows in an MC (Ca_d) on the average diameter of the aqueous droplets generated using hydrophobic stainless-steel MC array chips.

Table 3-1. Dimensions of stainless-steel MC arrays used in this study

Model code	MC depth h_{MC} (μm)	MC width w_{MC} (μm)	MC length l_{MC} (μm)	Terrace length $h_{terrace}$ (μm)	Totalchannel number (-)
SSMC-30	30	35	1400	300	22
SSMC-100	100	100	1500	400	22

Chapter 5
General conclusions and perspectives

5.1 Introduction

This study has focused on the generation of monodisperse W/O emulsions by MC arrays. In both direct and premix MCE, the hydrophobicity of MC array chip surfaces is an important factor affecting the droplet generation, because the preparation of W/O emulsions needs a totally hydrophobic MC array chip surface. The dimensions of each MC array chip mainly determine the resulting droplet size. In direct MCE, the large aqueous droplets were successfully produced using hydrophobic straight-through stainless-steel MC array chips. The droplet size and size distribution, flow rate of dispersed phase, and capillary number were analyzed in direct MCE. In premix MCE, the effects of operating pressure, emulsifier and PEG concentrations, and water phase ratio on the droplet generation and droplet size distribution were analyzed. In addition, the droplet break-up was analyzed by using grooved premix MCE.

5.2 Summary of each chapter

Chapter 1

Fundamentals of emulsion, application, stability of emulsions, emulsification techniques, andhydrophobicity of MC array chips surfaces, contact angle were introduced in chapter 1. The objectives of this thesis were also described.

Chapter 3

In this chapter, hydrophobically treated stainless-steel MC array chips were used for preparing monodisperse W/O emulsions. A water-saturated decane containing 5 wt% tetraglycerin monolaurate condensed ricinoleic acid ester was used as the continuous

phase. A Milli-Q water containing 5 wt% polyethylene glycol (M. W. 20,000) and 5 wt% NaCl was used as the dispersed phase. The stainless-steel MC array chips used for direct MCE had a sufficiently high contact angle of the dispersed phase to their surface in the continuous phase. The resultant uniform-sized aqueous droplets with coefficient of variation of <5% had average diameters of 100 to 300 μm, depending on the MC cross-sectional size. The maximum productivity of uniform-sized aqueous droplets reached higher than 1 mL/h. The difference in the critical capillary number of the dispersed phase that flows in a 100-μm depth MC was 1.5 times greater than that in a 30 μm depth MC.

5.3 General conclusions

- Hydrophobic stainless-steel MC chips were capable of preparing monodisperse W/O emulsions by direct MCE.
- Aqueous droplet size was affected by channel depth of stainless-steel MC array chip by direct MCE.

5.4 Perspectives

The interest in monodisperse W/O emulsions produced by MCE has been greatly increased in the past two decades. The highly monodisperse droplets can be produced by direct MCE. The large-scale production of monodisperse single and multiple emulsions are also highly expected. The increasing applications of W/O emulsions in various fields (food, cosmetic, and chemical industries) make us believe that the large-scale production is necessary. It is expected that the droplet size of monodisperse W/O emulsions by direct

MCE could be larger which can be used to some value-added products. Monodisperse W/O emulsions with fine droplets can also be used to prepare W/O/W emulsions which are normally used for food production. In addition, the productivity of monodisperse W/O emulsions should be larger, which can help these devices for industrial applications. It is expected that the direct MCE and premix MCE can be large-scale used in the food industry and focus on W/O emulsion-based food production.

The research findings of this thesis are also helpful for future applications in the hydrophobic properties of MC array chips. Further investigation is need to analyze the hydrophobicity of different MC array chips, which is better for large-scale industrial applications. The findings of the effect of operating conditions (e.g. operating pressure, emulsifier concentration, and viscosity of the two phases) are useful on the further study about long-term production of W/O emulsions by premix MCE. It is very important to understand the behaviors of W/O emulsions by premix MCE, leading to potential application of the W/O emulsion-based production in food industry.

There are some items necessary to investigate in the future research, such as how to keep the hydrophobicity of MC array chips for long term and how to continuously produce continuous W/O emulsion-based products by using premix MCE.

Literatures Cited

Andresen, M., & Stenius, P. (2007). Water-in-oil emulsions stabilized by hydrophobized microfibrillated cellulose. Journal of Dispersion Science and Technology, 28(6), 837-844.

Abrahamse, A. J., Van Lierop, R., Van der Sman, R. G. M., Van der Padt, A., & Boom, R. M. (2002). Analysis of droplet formation and interactions during cross-flow membrane emulsification. Journal of Membrane Science, 204(1), 125-137.

Anna, S. L., Bontoux, N., & Stone, H. A. (2003). Formation of dispersions using "flow focusing" in microchannels. Applied Physics Letters, 82(3), 364-366.

Ax, K., Mayer-Miebach, E., Link, B., Schuchmann, H., & Schubert, H. (2003). Stability of lycopene in oil-in-water emulsions. Engineering in Life Sciences, 3(4), 199-201.

Bibette, J. (1991). Depletion interactions and fractionated crystallization for polydisperse emulsion purification. Journal of Colloid and Interface Science, 147(2), 474-478.

Brouzes, E., Medkova, M., Savenelli, N., Marran, D., Twardowski, M., Hutchison, J. B.& Samuels, M. L. (2009). Droplet microfluidic technology for single-cell high-throughput screening. Proceedings of the National Academy of Sciences, 106(34), 14195-14200.

Fujiu, K. B., Kobayashi, I., Neves, M. A., Uemura, K., & Nakajima, M. (2011). Effect of temperature on production of soybean oil-in-water emulsions by microchannel emulsification using different emulsifiers. Food Science and Technology

Research, 17(2), 77-86.

Fujiu, K. B., Kobayashi, I., Neves, M. A., Uemura, K., & Nakajima, M. (2012). Influence of temperature on production of water-in-oil emulsions by microchannel emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 411, 50-59.

Charcosset, C., Limayem, I., & Fessi, H. (2004). The membrane emulsification process—a review. Journal of Chemical Technology and Biotechnology, 79(3), 209-218.

Charcosset, C. (2009). Preparation of emulsions and particles by membrane emulsification for the food processing industry. Journal of Food Engineering, 92(3), 241-249.

Cheng, C. J., Chu, L. Y., Zhang, J., Zhou, M. Y., & Xie, R. (2008). Preparation of monodisperse poly (N-isopropylacrylamide) microspheres and microcapsules via Shirasu-porous-glass membrane emulsification. Desalination, 234(1-3), 184-194.

Chuah, A. M., Kuroiwa, T., Kobayashi, I., Zhang, X., & Nakajima, M. (2009). Preparation of uniformly sized alginate microspheres using the novel combined methods of microchannel emulsification and external gelation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 351(1), 9-17.

Chu, L. Y., Park, S. H., Yamaguchi, T., & Nakao, S. I. (2001). Preparation of thermo-responsive core-shell microcapsules with a porous membrane and poly (N-

isopropylacrylamide) gates. Journal of Membrane Science, 192(1), 27-39.

Djenouhat, M., Hamdaoui, O., Chiha, M., & Samar, M. H. (2008). Ultrasonication-assisted preparation of water-in-oil emulsions and application to the removal of cationic dyes from water by emulsion liquid membrane: Part 1: Membrane stability. Separation and Purification Technology, 62(3), 636-641.

Dowding, P. J., Goodwin, J. W., & Vincent, B. (2001). Production of porous suspension polymer beads with a narrow size distribution using a cross-flow membrane and a continuous tubular reactor. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 180(3), 301-309.

Fujiu, K. B., Kobayashi, I., Neves, M. A., Uemura, K., & Nakajima, M. (2012). Influence of temperature on production of water-in-oil emulsions by microchannel emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 411, 50-59.

Giovambattista, N., Debenedetti, P. G., & Rossky, P. J. (2007). Effect of surface polarity on water contact angle and interfacial hydration structure. The Journal of Physical Chemistry B, 111(32), 9581-9587.

Gijsbertsen-Abrahamse A J (2003) Membrane Emulsification: Process Principles. PhD Thesis Wageningen University.

Gu, Y., & Li, D. (2000). The ζ-potential of glass surface in contact with aqueous solutions. Journal of Colloid and Interface Science, 226(2), 328-339.

Hatate, Y., Uemura, Y., Ijichi, K., Kato, Y., Hano, T., Baba, Y., & Kawano, Y. (1995). Preparation of GPC packed polymer beads by a SPG membrane

emulsifier. Journal of Chemical Engineering of Japan, 28(6), 656-659.

Hardt S, Schonfeld F, Weise F, Hoffmann C, Hessel V, Ehrfeld W (2001) ProReferences 171 ceedings of the 4th International Conference on Modeling and Simulations of Microsystems 2011, Hilton Head Island, SC, 233-226.

Hunter R J (1986) Foundations of Colloid Sciences. Vol 1 Oxford University Press, Oxford 752.

Israelachvili J J (1992) Intermolecular and surface forces. Academic press. London, UK.

Iwamoto, S., Nakagawa, K., Sugiura, S., & Nakajima, M. (2002). Preparation of gelatin microbeads with a narrow size distribution using microchannel emulsification. AAPS Pharmscitech, 3(3), 72-76.

Joscelyne, S. M., & Trägårdh, G. (2000). Membrane emulsification—a literature review. Journal of Membrane Science, 169(1), 107-117.

Joseph, S., & Bunjes, H. (2013). Influence of membrane structure on the preparation of colloidal lipid dispersions by premix membrane emulsification. International Journal of Pharmaceutics, 446(1), 59-62.

Katoh, R., Asano, Y., Furuya, A., Sotoyama, K., & Tomita, M. (1996). Preparation of food emulsions using a membrane emulsification system. Journal of Membrane Science, 113(1), 131-135.

Katoh, R., Asano, Y., Furuya, A., Sotoyama, K., Tomita, M., & Okonogi, S. (1997). A method of preventing dispersed droplets from crushing using a membrane emulsification system. Journal of the Japanese Society for Food Science and Technology-nippon Shokuhin Kagaku Kogaku Kaishi 44(3), 233-237.

Kawakatsu, T., Kikuchi, Y., & Nakajima, M. (1997). Regular-sized cell creation in microchannel emulsification by visual microprocessing method. Journal of The American Oil Chemists' Society, 74(3), 317-321.

Kawakatsu T, Trägårdh G, Trägårdh C. Production of W/O/W emulsions and S/O/W pectin microcapsules by microchannel emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2001, 189(1): 257-264.

Kawakatsu, T., et al. The effect of the hydrophobicity of microchannels and components in water and oil phases on droplet formation in microchannel water-in-oil emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects 179 (2001): 29-37.

Kawakatsu, T., Komori, H., Nakajima, M., Kikuchi, Y., & Yonemoto, T. (1999). Production of monodispersed oil-in-water emulsion using crossflow-type silicon microchannel plate. Journal of Chemical Engineering of Japan, 32(2), 241-244.

Khalid, N., Kobayashi, I., Neves, M. A., Uemura, K., Nakajima, M., & Nabetani, H. (2016). Microchannel emulsification study on formulation and stability characterization of monodisperse oil-in-water emulsions encapsulating quercetin. Food Chemistry, 212, 27-34.

Khalid, N., Kobayashi, I., Wang, Z., Neves, M. A., Uemura, K., Nakajima, M., & Nabetani, H. (2015). Formulation characteristics of triacylglycerol oil-in-water emulsions loaded with ergocalciferol using microchannel emulsification. RSC Advances, 5(118), 97151-97162.

Kobayashi I, Mukataka S, Nakajima M. Production of monodisperse oil-in-water emulsions using a large silicon straight-through microchannel plate. Industrial & Engineering Chemistry Research, 2005, 44(15): 5852-5856.

Kobayashi I, Nakajima M, Chun K, et al. Silicon array of elongated through-holes for monodisperse emulsion droplets. AIChE Journal, 2002, 48(8): 1639-1644.

Kobayashi I, Nakajima M, Mukataka S. Preparation characteristics of oil-in-water emulsions using differently charged surfactants in straight-through microchannel emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2003, 229(1): 33-41.

Kobayashi, I., Hirose, S., Katoh, T., Zhang, Y., Uemura, K., & Nakajima, M. (2008). High-aspect-ratio through-hole array microfabricated in a PMMA plate for monodisperse emulsion production. Microsystem Technologies, 14(9-11), 1349-1357.

Kobayashi, I., Mukataka, S., & Nakajima, M. (2005). Novel asymmetric through-hole array microfabricated on a silicon plate for formulating monodisperse emulsions. Langmuir, 21(17), 7629-7632.

Kobayashi, I., Kunihiko Uemura, and Nakajima M. (2008). Breakup of Oil-in-Water Emulsion Droplets in Microchannel Array Devices at Low Applied Pressures. Journal of Chemical Engineering of Japan 41: 420-426.

Kobayashi, I., Murayama, Y., Kuroiwa, T., Uemura, K., & Nakajima, M. (2009). Production of monodisperse water-in-oil emulsions consisting of highly

uniform droplets using asymmetric straight-through microchannel arrays. Microfluidics and Nanofluidics, 7(1), 107.

Kobayashi, I., Vladisavljević, G. T., Uemura, K., & Nakajima, M. (2011). CFD analysis of microchannel emulsification: Droplet generation process and size effect of asymmetric straight flow-through microchannels. Chemical Engineering Science, 66(22), 5556-5565.

Kobayashi, I., Wada, Y., Hori, Y., Neves, M. A., Uemura, K., & Nakajima, M. (2012). Microchannel Emulsification Using Stainless-Steel Chips: Oil Droplet Generation Characteristics. Chemical Engineering & Technology, 35(10), 1865-1871.

Kukizaki, M., & Goto, M. (2007). Preparation and characterization of a new asymmetric type of Shirasu porous glass (SPG) membrane used for membrane emulsification. Journal of Membrane Science, 299(1), 190-199.

Katoh, Ryoh, et al. Preparation of food emulsions using a membrane emulsification system. Journal of Membrane Science 113 (1996): 131-135.

Kikuchi, Y., Sato, K., & Mizuguchi, Y. (1994). Modified cell-flow microchannels in a single-crystal silicon substrate and flow behavior of blood cells. Microvascular research, 47(1), 126-139.

Kawakatsu, T., Komori, H., Nakajima, M., Kikuchi, Y., & Yonemoto, T. (1999). Production of monodispersed oil-in-water emulsion using crossflow-type silicon microchannel plate. Journal of Chemical Engineering of Japan, 32(2), 241-244.

Kawakatsu, T., Kikuchi, Y., & Nakajima, M. (1996). Visualization of microfiltration phenomena using microscope video system and silicon microchannels. Journal of Chemical Engineering of Japan, 29(2), 399-401.

Kawakatsu, T., Trägårdh, G., & Trägårdh, C. (2001). Production of W/O/W emulsions and S/O/W pectin microcapsules by microchannel emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 189(1), 257-264.

Kandori, K., Kishi, K., & Ishikawa, T. (1991). Formation mechanisms of monodispersed W/O emulsions by SPG filter emulsification method. Colloids and Surfaces, 61, 269-279.

Katoh, R., Asano, Y., Furuya, A., Sotoyama, K., & Tomita, M. (1996). Preparation of food emulsions using a membrane emulsification system. Journal of Membrane Science, 113(1), 131-135.

Kikuchi, Y., Sato, K., Ohki, H., & Kaneko, T. (1992). Optically accessible microchannels formed in a single-crystal silicon substrate for studies of blood rheology. Microvascular Research, 44(2), 226-240.

Köster S, Angilė F E, Duan H, Agresti JJ, Wintner A, Schmitz C, Rowat A C, Merten C A, Pisignano D, Groffiths A D, Weitz D A (2008) Drop-base microfluidic devices for encapsulation of single cells. Lab Chip (8), 1110-1115.

Link, D. R., Anna, S. L., Weitz, D. A., & Stone, H. A. (2004). Geometrically mediated breakup of drops in microfluidic devices. Physical Review Letters, 92(5), 054503.

Lucassen-Reynders, E. H., & Kuijpers, K. A. (1992). The role of interfacial properties in

emulsification. Colloids and Surfaces, 65(2-3), 175-184.

McClements, D. J. (2015). Food emulsions: principles, practices, and techniques. CRC press.

Murshed, S. S., Tan, S. H., Nguyen, N. T., Wong, T. N., & Yobas, L. (2009). Microdroplet formation of water and nanofluids in heat-induced microfluidic T-junction. Microfluidics and Nanofluidics, 6(2), 253-259.

Menon, V. B., & Wasan, D. T. (1988). Characterization of oil—water interfaces containing finely divided solids with applications to the coalescence of water-in-oil Emulsions: A review. Colloids and Surfaces, 29(1), 7-27.

Muijlwijk, K., Berton-Carabin, C., & Schroën, K. (2016). Cross-flow microfluidic emulsification from a food perspective. Trends in Food Science & Technology, 49, 51-63.

Nakashima, T., Shimizu, M., & Kukizaki, M. (2000). Particle control of emulsion by membrane emulsification and its applications. Advanced Drug Delivery Reviews, 45(1), 47-56.

Nakashima, T., Shimizu, M., & Kukizaki, M. (1991). Membrane emulsification operation manual. Department of Chemistry, Industrial Research Institute of Miyazaki Prefecture, Miyazaki.

Nakashima, T., Shimizu, M., & Kukizaki, M. (2000). Particle control of emulsion by membrane emulsification and its applications. Advanced Drug Delivery Reviews, 45(1), 47-56.

Nakashima, T., Shimizu, M., & Kukizaki, M. (1992). Membrane emulsification by

microporous glass. In Key Engineering Materials (Vol. 61, pp. 513-516). Trans Tech Publications.

Nazir, A., Schroën, K., & Boom, R. (2011). High-throughput premix membrane emulsification using nickel sieves having straight-through pores. Journal of Membrane Science, 383(1), 116-123.

Nakaya, K., Kohata, T., Doisaki, N., Ushio, H., & Ohshima, T. (2006). Effect of oil droplet sizes of oil-in-water emulsion on the taste impressions of added tastants. Fisheries Science, 72(4), 877-883.

Nisisako, T., Torii, T., & Higuchi, T. (2004). Novel microreactors for functional polymer beads. Chemical Engineering Journal, 101(1), 23-29.

Nisisako, T., & Torii, T. (2008). Microfluidic large-scale integration on a chip for mass production of monodisperse droplets and particles. Lab on a Chip, 8(2), 287-293.

Neves, M. A., Ribeiro, H. S., Kobayashi, I., & Nakajima, M. (2008). Encapsulation of lipophilic bioactive molecules by microchannel emulsification. Food Biophysics, 3(2), 126-131.

Neves, M. A., Ribeiro, H. S., Fujiu, K. B., Kobayashi, I., & Nakajima, M. (2008). Formulation of controlled size PUFA-loaded oil-in-water emulsions by microchannel emulsification using β-carotene-rich palm oil. Industrial & Engineering Chemistry Research, 47(17), 6405-6411.

Okochi H, Nakano M (1997) Comparative study of two preparation method of w/o/w emulsions: stirring and membrane emulsification. Chem Pharm Bull 45:1323-

1326.

Okushima, S., Nisisako, T., Torii, T., & Higuchi, T. (2004). Controlled production of monodisperse double emulsions by two-step droplet breakup in microfluidic devices. Langmuir, 20(23), 9905-9908.

Peng, S. J., & Williams, R. A. (1998). Controlled production of emulsions using a crossflow membrane: Part I: Droplet formation from a single pore. Chemical Engineering Research and Design, 76(8), 894-901.

Ribeiro, H. S., Rico, L. G., Badolato, G. G., & Schubert, H. (2005). Production of O/W emulsions containing astaxanthin by repeated premix membrane emulsification. Journal of Food Science, 70(2), E117–E123.

Schröder, V., Behrend, O., & Schubert, H. (1998). Effect of dynamic interfacial tension on the emulsification process using microporous, ceramic membranes. Journal of Colloid and Interface Science, 202(2), 334-340.

Sugiura, S., Nakajima, M., Tong, J., Nabetani, H., &Seki, M. (2000). Preparation of monodispersed solid lipid microspheres using a microchannel emulsification technique. Journal of Colloid and Interface Science, 227(1), 95-103.

Sugiura, S., Nakajima, M., Ushijima, H., Yamamoto, K., & Seki, M. (2001). Preparation characteristics of monodispersed water-in-oil emulsions using microchannel emulsification. Journal of Chemical Engineering of Japan, 34(6), 757-765.

Sugiura, S., Kuroiwa, T., Kagota, T., Nakajima, M., Sato, S., Mukataka, S.& Ichikawa, S. (2008). Novel method for obtaining homogeneous giant vesicles from a

monodisperse water-in-oil emulsion prepared with a microfluidic device. Langmuir, 24(9), 4581-4588.

Sugiura, S., Nakajima, M., & Seki, M. (2002). Effect of channel structure on microchannel emulsification. Langmuir, 18(15), 5708-5712.

Sugiura, S., Nakajima, M., Iwamoto, S., & Seki, M. (2001). Interfacial tension driven monodispersed droplet formation from microfabricated channel array. Langmuir, 17(18), 5562-5566.

Sugiura, S., Nakajima, M., Kumazawa, N., Iwamoto, S., & Seki, M. (2002). Characterization of spontaneous transformation-based droplet formation during microchannel emulsification. The Journal of Physical Chemistry B, 106(36), 9405-9409.

Sugiura, S., Nakajima, M., Ushijima, H., Yamamoto, K., & Seki, M. (2001). Preparation characteristics of monodispersed water-in-oil emulsions using microchannel emulsification. Journal of Chemical Engineering of Japan, 34(6), 757-765.

Tan, C., Kobayashi, I., & Nakajima, M. (2005, December). Preparation of monodispersed refined-bleached-deodorized (RBO) palm olein-in-water emulsions by microchannel emulsification. In Proceedings of the International Chemical Congress of Pacific Basin Societies (pp. 15-20).

Tong, J., Nakajima, M., Nabetani, H., & Kikuchi, Y. (2000). Surfactant effect on production of monodispersed microspheres by microchannel emulsification method. Journal of Surfactants and Detergents, 3(3), 285-293.

Tong, J., Nakajima, M., Nabetani, H., Kikuchi, Y., & Maruta, Y. (2001). Production of oil-in-water microspheres using a stainless steel microchannel. Journal of Colloid and Interface Science, 237(2), 239-248.

Trentin, A., Ferrando, M., López, F., & Güell, C. (2009). Premix membrane O/W emulsification: Effect of fouling when using BSA as emulsifier. Desalination, 245(1-3), 388-395.

Vladisavljević, G. T., & Schubert, H. (2003). Influence of process parameters on droplet size distribution in SPG membrane emulsification and stability of prepared emulsion droplets. Journal of Membrane Science, 225(1), 15-23.

Vladisavljević G. T. & Williams R A. (2006). Manufacture of large uniform droplets using rotating membrane emulsification. Journal of Colloid and Interface Science, 299(1): 396-402.

Vladisavljević, G. T., Kobayashi, I., & Nakajima, M. (2012). Production of uniform drop lets using membrane, microchannel and microfluidic emulsification devices. Microfluidics and Nanofluidics, 13(1), 151-178.

Vladisavljević, G. T., Tesch, S., & Schubert, H. (2002). Preparation of water-in-oil emulsions using microporous polypropylene hollow fibers: influence of some operating parameters on droplet size distribution. Chemical Engineering and Processing: Process Intensification, 41(3), 231-238.

Vladisavljevic, G. T., & Schubert, H. (2002). Preparation and analysis of oil-in-water emulsions with a narrow droplet size distribution using Shirasu-porous-glass (SPG) membranes. Desalination, 144(1), 167-172.

Vladisavljević, G. T., Shimizu, M., & Nakashima, T. (2004). Preparation of monodisperse multiple emulsions at high production rates by multi-stage premix membrane emulsification. Journal of Membrane Science, 244(1), 97-106.

Williams, R. A., Peng, S. J., Wheeler, D. A., Morley, N. C., Taylor, D., Whalley, M., & Houldsworth, D. W. (1998). Controlled production of emulsions using a crossflow membrane: part II: industrial scale manufacture. Chemical Engineering Research and Design, 76(8), 902-910.

Xu, J. H., Li, S. W., Tan, J., Wang, Y. J., & Luo, G. S. (2006). Controllable preparation of monodisperse O/W and W/O emulsions in the same microfluidic device. Langmuir, 22(19), 7943-7946.

Xu, S., Nie, Z., Seo, M., Lewis, P., Kumacheva, E., Stone, H. A.& Whitesides, G. M. (2005). Generation of monodisperse particles by using microfluidics: control over size, shape, and composition. Angewandte Chemie, 117(5), 734-738.

Yamazaki, N., Naganuma, K., Nagai, M., Ma, G. H., & Omi, S. (2003). Preparation of W/O (Water-in-Oil) Emulsions Using a PTFE (Polytetrafluoroethylene) Membrane—A New Emulsification Device. Journal of Dispersion Science and Technology, 24(2), 249-257.

Yang, J., Hao, D. X., Bi, C. X., Su, Z. G., Wang, L. Y., & Ma, G. H. (2010). Rapid synthesis of uniform magnetic microspheres by combing premix membrane emulsification and in situ formation techniques. Industrial & Engineering Chemistry Research, 49(13), 6047-6053.

Zanatta, V., Rezzadori, K., Penha, F. M., Zin, G., Lemos-Senna, E., Petrus, J. C. C., & Di Luccio, M. (2017). Stability of oil-in-water emulsions produced by membrane emulsification with microporous ceramic membranes. Journal of Food Engineering, 195, 73-84.

Zhou, Q. Z., Ma, G. H., & Su, Z. G. (2009). Effect of membrane parameters on the size and uniformity in preparing agarose beads by premix membrane emulsification. Journal of Membrane Science, 326(2), 694-700.

Acknowledgments

First of all, I would like to express my profound appreciation and gratitude to my doctor supervisor, Professor Mitsutoshi Nakajima for his excellent support and guidance. I feel grateful to him not only the chance given to me to study at University of Tsukuba, but also the opportunities given to me to attend the meetings and conferences, which have broaden my views.

I feel grateful to Prof. Isao Kobayashi for his valuable guidance during all my study, for his untiring and constant support. His infinite patience help me to develop my research and give me so many helpful comment and advice in my doctor thesis.

My appreciation is to Prof. Kunihiko Uemura for all his kind and helpful advice in my study and life in the laboratory.

I am indebted to Prof. Marcos Antouio das Neves for all his kindness and advice in my research and daily life.

I feel grateful to Dr. Yanru Zhang for her support in my research and helpful advice in my thesis. She gives me so many help in study and life in the laboratory.

My heartfelt gratitude is to the past and present members in the Food Resources Engineering laboratory for their help and support: Dr. Hiroyuki Kozu, Dr. Nauman Khalid, Ms. Akane Yasubaga, Ms Ayani Makisita, Mr. Sota Matsumoto, Ms. Kaoru Abe, Mr. Gaofeng Shu, Mr. Yiguo Zhao, Mr. Yuxin Wang, Mr. Yifeng Jiang, Ms. Lara, Grace, Mr. Muhammd, Arif and all other laboratory members. I am indebted to Mrs. Mitsuko Kawamoto, Mrs. Takahashi for all their kind support and help in the lab.

My appreciation is to my friends for their kind help and encouragement in my study and life.

Finally, I would like to dedicate this dissertation to my husband, my parents and my brother for their unconditional support, encouragement, love, trust and dedication to me.

June, 2017

Ran LI