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Effect of Junction Geometry on Monodispersed Microdroplet Generation in Microfluidic Aqueous Two-Phase Systems

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EFFECT OF JUNCTION GEOMETRY ON MONODISPERSED MICRODROPLET GENERATION IN MICROFLUIDIC AQUEOUS TWO-PHASE SYSTEMS

by

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ABSTRACT

EFFECT OF JUNCTION GEOMETRY ON MONODISPERSED MICRODROPLET GENERATION IN MICROFLUIDIC AQUEOUS TWO-PHASE SYSTEMS

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Under the Supervision of Professor Woo-Jin Chang

Aqueous two-phase system (ATPS) consists of two immiscible water-based solutions of polymers, which can form phase partitioning. Dextran and polyethylene glycol I used in this thesis is the one of common components of aqueous two-phase system give a reliable and incompatible environment for purification of biomedical products and cellular macromolecules. Recently, ATPS have received increasing attention as a separation method in microfluidic device due to the advantages of biocompatibility, unlimited combination, and low interfacial tension. Hence, it became an important to discover researches related to ATPS microfluidic device.

Microdroplets produced in microfluidic device are a largely interesting phenomenon for various applications. Monodisperse and size manageable microdroplets using ATPS could potentially be used to better micro-enviornment. However, extremely low interfacial tension (≤ 100 μN/m) leading to viscoelastic fluid (non-Newtonian) characteristic makes it difficult to generate microdroplets. It is necessary to control the physical and topological behavior of ATPS.

Therefore, this thesis aims to study fluid mechanism for droplet-based microfluidics using ATPS. Droplet generation using aqueous two phase systems (ATPS) in microfluidic device was studied by various junction areas which were considered as T-junction, flow-focusing, and double-flow-focusing. The characteristic of low interfacial tension and high viscosity between aqueous phases was the challenge to produce uniform micro-droplets.

The importance of this experiment is that in contrast to another external installations previously studied, double-flow-focusing channel drew advantages of simple method, cost effective, and heavy workload. Without the continuous mechanical pressure by pressure-driven flow, no external actuations were used. T-junctions and flow-focusing, broadly used for microfluidic device, were compared with double-flow-focusing channel. The role of each flow-focusing junction for monodisperse water-in-water (w/w) droplets was investigated. Additional flow-focusing junction for monodisperse water-in-water (w/w) droplets brought the consequence different from T-juntion and flow-focusing. Moreover, I proved that PEG and Dextran droplets within double-flow-focusing could be formed with combination of two continuous flow rates. Surfactant impact on droplet generation in ATPS was studied.

Thus, a double-flow-focusing microfluidic device I developed was able to be a crucial method to generate water-in-water (w/w) droplets due to the stability of dispersion between two junction areas.

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LIST OF ABBREVIATIONS

ATPS	Aqueous Two-Phase System
Ca	Capillary Number
De	Deborah Number
DEX	Dextran
El	Elasticity Number
Gr	Grashof Number
Kn	Kundsen Number
MEMS	Micro-Electro-Mechanical System
M.W.	Molecular weight
PDMS	Polydimethylsiloxane
Pe	Peclet Number
PEG	Poly(ethylene glycol)
Ra	Rayleigh Number
Re	Reynolds Number
We	Weber Number
Wi	Weissenberg Number
v/v	Volume per volume
w/o	Water-in-oil
w/w	Water-in-water
w/w	Weight per volume

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I would also like to thank Dr. Chang's patience and guidance towards me. His desire and enthusiasm towards cutting-edge science has always been a motivation for me. Apart from his encouragement and support in my research, I have learned many valuable philosophies in life, which will benefit me in the future as a scientist.

Chapter 1

Introduction

The first chapter will briefly introduce background information related to the research of aqueous two-phase system in droplet-based microfluidic device. First of all, the improvement for the microfluidic technology and its current application will be explained. It has a wide range of applications in biological process and traditional chemical processes with various advantages. The following section will be expanded to the droplet-based microfluidic system of interests in this thesis. Finally, the characteristics of aqueous two-phase system (ATPS) will be also discussed with its applications in microfluidic device.

1.1 Introduction to Microfluidics

In the past few decades, MEMS (Micro-Electro-Mechanical System) technology has paid attention due to increasing public demand on more sophisticated and minimized equipment and devices. Under the supervision of cost minimization, an initial concept of

manufacturing a biochemical laboratory on a small chip resulted in a microfluidic device. When microfluidics came out as a new alternative one in micro/nanotechnology, it has become revolution forward the existing chemical and biological processes. Microfluidics is a multidisciplinary science of designing, manufacturing, and formulating devices and processes that lead to change our ability to manipulate tiny volumes of fluid or micro and nanoparticles. Currently, it is not only used for chemical and biochemical analysis [1], but also for chemical synthesis [2][3], sensors [4][5], cell capture and counting, micropumps, actuators, and high-throughput design [1][6]. Since the early 1990s, there has been a progressive development in microfluidic field enabled by the standardized technologies based on MEMS such as photolithography. The most recent soft lithography techniques provide a well-standardized procedure for microfluidic devices fabrication [7].

The microfluidic device is a small chip containing microchannels, inlets, and outlets. For a typical microfluidic system, the fluid is introduced into the microchannels *via* the inlets and is transported along the microchannels until the outlet. The most common means of driving fluids passing through microscale-diameter tubes is pressure-driven force applied by the use of pressurized syringe pumps or by electric field.

The advantage of microfluidics is its small volume consumption, fast analysis and respond, sophisticated control, compact size, high-throughput monitor, low fabrication cost, and environmental material. From the view of fluid mechanics, it is important for micro-scale studying to understand interfacial phenomena that physical factors, which are interfacial tension, pressure drop, and viscosity, are dominant rather than the effect of gravity. Micro-scale flows are typically laminar flow due to short length scales.

1.2 Droplet-based Microfluidics

Droplet-based microfluidics involves the generation, manipulation, and the use of discrete picoliter to nanoliter droplets inside microfluidic devices. The demand of droplets as micro-reactors that manipulated small volume for biological and chemical analysis has been increased in the ability of lab-on-a-chip applications [8]. The studies of observing chemical and biochemical reactions in droplets have been also explored in microfluidic devices [9][10]. Since the perception of miniaturization became significant for biological and chemical sciences, water-in-oil (w/o) microdroplets has brought advantages of a lower cost and simple experimental format for high-throughput screening. Hence, these micro-reactors developed in a microfluidic device clearly expanded possibilities to deploy for biological and chemical processes. One faultless advantage of monodispersed droplets is that very small volumes and large amounts of individual reactors allow the encapsulation and the analysis of DNA, protein, or cells [11].

Because of the economy of small volumes and ability for high numbers, droplet generation in microfluidic channel will have to be versatile and flexible so that micro-droplet as single reactor can detect oncogenes or other important disease genes in a high-throughput screening. In well-known channel geometries such as the T-shaped junctions and Flow-focusing, two immiscible fluids, when they meet each other, formulate droplets of uniform size. Each micro-size droplet serves an independent reactor with chaotic advection. Various analytical methods have been integrated on a microfluidic device [1].

In summary, previous studies for aqueous droplet's application are conducted by using biocompatible oils and surfactants so that cells and worms can last their lives in droplets [12]. However, it is able to be a more effective solution for quantitative cell biology and

enzyme/protein separation to provide individual microenvironment in aqueous two-phases system.

Importantly, the field of droplet microfluidics has relied on the use of organic solvents in combination with aqueous solutions for the production of particles form emulsions. A disadvantage of the use of organic solutions is that they harm biomaterial upon encapsulation. Aqueous biodegradable polymer microdroplet not only apply for a better understanding of cells, but these biodegradable materials looks forward to being practical solutions in fields as diverse as food, cosmetics, pharmacy, self-assembly [13], tissue engineering[14], multiplexing assays [15], and drug delivery [16].

1.3 Aqueous Two-Phase System

It is very difficult to completely isolate the desired products from organic solvents, which are mostly very toxic. However, Aqueous Two-Phase System (ATPS) has become a powerful method for separation of a range of biomaterials, such as DNA, protein, cell, organelle, and biological membrane [17]. Generally, ATPS is formed by the incompatibility of two aqueous polymer solutions [18] or one polymer and an appropriate salt solution [19][20]. The phase separation occurs above certain concentrations of polymers or salts in the system because of the repulsion between molecules at high concentrations.

Normally, approximately 65 ~ 95% of high water content in ATPS gives favorable condition to the stability of biomolecules compared to water/organic two-phase systems [21]. Also, various different separation environments are available in ATPS depend on the concentration and molecular weight of the composing salt and polymers,

temperature, and pH. Recently, aqueous two-phase systems (ATPS) have been extensively studied due to the fact that they do not contain harmful chemicals. One typical sample is an aqueous two-phase system composed of poly(ethylene glycol) (PEG) and dextran (DEX). It has been found this ATPS is able to separate a variety of macromolecules, membranes, and organelles, and cells [22][23].

Moreover, a picoliters of volume of ATPS microenvironment for biochemical reaction makes it possible to mimic heterogeneous environment inside a eukaryotic cell in microfluidics channels. One of the characteristics of such aqueous two-phase systems is that their interfacial tension is extremely low. The typical range of interfacial tension in ATPS is from 1 to a few hundred μN/m [24][25]. In this project, microfluidic aqueous two-phase micro-droplet systems composed of PEG and DEX solutions have been successfully created and investigated.

Chapter 2

Literature Survey

This chapter will summarize the physical understanding, main observation of droplet formation, aqueous two-phase system in droplet-based microfluidics, and its applications in biology, chemistry, and biomedicine [28]. To begin with, section 2.1 reviews the physics of small size world, which explain a series of dimensionless numbers related to various physical phenomena. Section 2.1.2 introduces governing differential equations for fluid dynamics. Specifically, the Reynolds number, *Re*, representing inertial effects, and the capillary number, *Ca*, expressing the importance of interfacial tension are discussed (section 2.1.3 and section 2.1.4). In this literature survey, we deal with the production of picoliter droplets by microfluidic method (section 2.2). Because of low Reynolds number and Capillary number, the laminar flow in microfluidic devices allows the mass production of microparticles with size control, shape, and morphology. Droplet-based microfluidic system primarily relies on the use of oil and water for the formation of monodisperse particles (section 2.2.1) and has potential application for chemistry and

biology (section 2.2.2). In this thesis, we investigate the advantages of the use of immiscible aqueous solutions instead of oil and water systems (section 2.3). After providing background information on ATPS, we will assess the consequences of their use in microfluidics and one in droplets-based microfluidics the following sections 2.3.1 and 2.3.2. In contract to oil-water systems, the droplet formation produced by ATPS is a challenge because one of unique features of ATPS, which has extremely low interfacial tension.

2.1 Physics at the Micrometric Scale

The characteristic of microfluidics is that the fundamental physical properties, which are interfacial tension, pressure drop, and viscosity, are more significant factors than the effect of gravity as the size scale is decreased [26]. The interpretation of fluid physics in a small scale can be explained by relationships between various phenomena [27]. A series of dimensionless numbers express their relative importance meanings. Microfluidics has the potential dealing with small amount of volumes, fast analysis and response times, and high-throughput analysis in a short time [28]. Microfluidic device would be a powerful tool for better understanding microscale phenomena, which can implement experiments not possible on the macroscale. The recent attention of interest in fluid flows, and their manipulation and control, has been raised in micrometric scale [10]. Hence, it is significant to understand fluid dynamics where viscous effects, as a role of frictional influence interior to the fluid, are dominant in small length scales.

2.1.1 Dimensionless Numbers in Microfluidics

A wide range of physical phenomena takes place in microfluidic devices [27]. Dimensionless numbers defined as ratio of quantities that are not dimensionless can be used to express these phenomena of fluid mechanics in micro scale. In this perspective of fluid mechanics, we review the dimensionless numbers listed in Table 1 [29]: the Reynolds number, *Re*, relating inertial forces to viscous forces and the capillary number *Ca*, relating viscous forces to surface tension; the Deborah, Weissenberg, and elasticity numbers *De*, *Wi*, and *El*, expressing elastic effects; the Grashof and Rayleigh numbers *Gr* and *Ra*, relating transport mechanisms in buoyancy-driven flows, the Knudsen number *Kn*, relating microscopic to macroscopic length scales, and *We*, relating fluid's inertia to surface tention. The velocity profile is a parabolic Poiseuille flow in parallel substrates from the vertical sidewalls of the microchannel. Through microchannels, the profile of velocity field is linear and the shear rate is proportional to the flow rate. The steady Poiseuille flow has no convective transport of momentum shown in Figure 1.

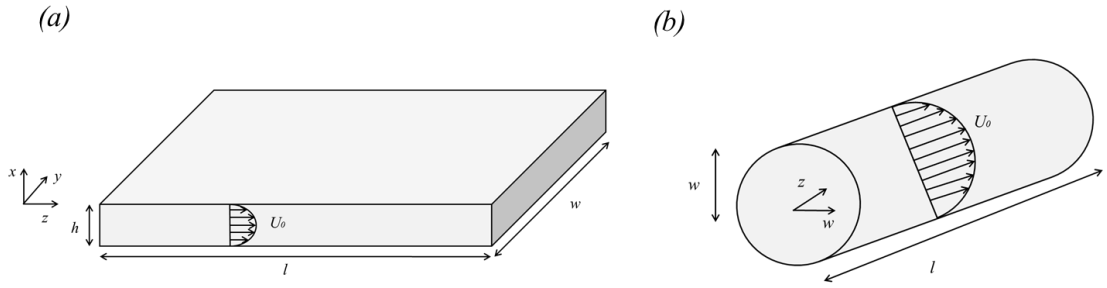


Figure 2.1 (a) rectangular and (b) circular microchannels, through which fluid flows with characteristic velocity scale U_0. Channel length will be denoted *l*, width (or radius) *w*, and height *h*. The coordinate *z* points downstream, *y* spans the width, and *x* spans the height.

Table [1] Dimensionless Numbers in Microfluidics

Dimensionless Number		Definition	Significance
Re	Reynolds No.	$\frac{\rho U_o L_o}{\mu}$	Inertia / viscous
Ca	capillary No.	$\frac{\mu U_o}{\gamma}$	Viscous / interfacial
We	Weber No.	$\frac{\rho U_0 l}{\sigma}$	Inertia / surface tension

2.1.2 The Continuity Equation and Navier-Stokes Equation

For flows in microfluidic device, aqueous solutions are considered as incompressible one having uniform density. This section describes the motion and deformation of fluids in micro scale. To begin with, we discuss conservation of mass and momentum for incompressible flows of Newtonian fluids. Conservation of mass is specified by the integral relation

$$\frac{\partial}{\partial t}\int_{CV} \rho dV = -\int_{Surface} (\rho\vec{\mathbf{u}}) \cdot \hat{\mathbf{n}} dA$$

where $\hat{\mathbf{n}}$ is a unit normal vector along the surfaces S [m^2], t is time [s], and ρ is the fluid density [kg/m^3]. This relation states that the change in mass within a control volume denoted by V [m^3] is given by the surface integral of the flux of mass crossing the surface of the volume. Conservation of mass equation can be written as;

$$\frac{\partial \boldsymbol{\rho}}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0$$

For an incompressible fluid ($\rho = constant$), this simplifies to

$$\nabla \cdot \vec{u} = 0$$

In microfluidic systems, the most dominant body force is the Coulomb force on the interface with net surface charge in an electric field [26]. Gravity force governing macroscopic fluid mechanics is negligible in microscale flows [30].

The conservation of momentum equation for a continuum is given by the Navier-Stokes equations if a fluid is Newtonian.

$$\rho\left(\frac{\partial \vec{\boldsymbol{u}}}{\partial t} + \vec{\boldsymbol{u}} \cdot \nabla \vec{\boldsymbol{u}}\right) = \nabla \cdot \vec{\boldsymbol{\sigma}} = -\nabla p + \mu \nabla^2 \vec{\boldsymbol{u}}$$

Forces on such elements are due to fluid stresses $\vec{\boldsymbol{\sigma}}$ (forces per unit area) applied on the element surfaces. However, many fluids exist for which the Newtonian formulation is inaccurate. Non-Newtonian fluids commonly used in microfluidics are made of long polymeric molecules, which align when they are sheared and slide along on another more easily at high strain rate [26]. In microfluidic device, many studies used long polymeric materials in separation media for DNA [31] and protein [32] separations. Colloidal systems (one example is blood) are also utilized [33]. Another non-Newtonian fluid type (one example is aqueous two-phase system (ATPS)) is a viscoelastic fluid, which combines a viscous (fluid) response with an elastic (solid) response [34]. Hence, other approaches are necessary for these non-Newtonian fluids that the stress-strain rate relation is non-linear in microfluidics device. Through this thesis, double-flow-focusing design we will explore could be a meaningful approach to observe of droplet breakup mechanism in aqueous two-phase system (ATPS) microfluidics.

2.1.3 The Reynolds Number, *Re*

Reynolds numbers is a useful characterization of flows in microchannel. In most microfluidic devices, the Reynolds number ranges between 10^{-6} - 10^{0} [29] because the channel length is less than 100 μm and flow velocity cm/sec. When Reynolds number is very small as resulting in linear, viscous forces typically overwhelm inertial forces, and the resulting flows are linear. Reynolds number is first defined by Osborne Reynolds (1842 ~ 1912) as follow:

$$Re = \frac{\rho U_o l}{\mu}$$

,where ρ is fluid density, U_o the characteristic velocity, l the length, and $\mu$the viscosity. Laminar flow depending on Reynolds number is occurred in microfluidics but inertia becomes less relevant to microfluidic systems. Inertia force has rarely impact on physics as systems are made ever smaller, however, many physical processes, such as capillary effects at free surfaces, viscoelasticity in polymer solutions, and electrokinetic effects, do exist.

2.1.4 The Capillary Number, *Ca*

Between two immiscible phases in micro scale, we can consider capillary stresses of magnitude γ/R balance viscous stresses μ/h, giving a characteristic droplet size

$$R \approx \frac{\gamma h}{\mu U_0} = \frac{h}{\mathrm{Ca}}$$

So, we have introduced the capillary number

$$Ca = \frac{\mu U_0}{\gamma}$$

, where γ is the surface tension [N/m], R the surface curvature [m], U_0 the characteristic velocity [m^2/s], and μ shear viscosity [$N \cdot s/m^2$]. A dimensionless parameter found whenever interfacial stresses compete with viscous stresses. The droplet size determines that surface tension of continuous phase act on interfacial area, and viscous stresses are stretched and pull the interface of disperse phase down into downstream. These stresses weaken the interfacial tension and form droplets [35][36]. Many pioneer studies are contributed for droplet breakup mechanism. A dispersed aqueous phase in flow-focusing did breakup relied on Rayleigh-Plateau instability [37-39]. Moreover, surface tension γ and viscosity μ are contributed to microfluidic flow when two immiscible fluids meet [40]. Thorsen, T. et al [41] examined firstly droplet formation at T-junction relied on Capillary number and Anna, S. L. et al [37] identified the monodispersity of jets at flow-focusing channel as shown in Figure 2.

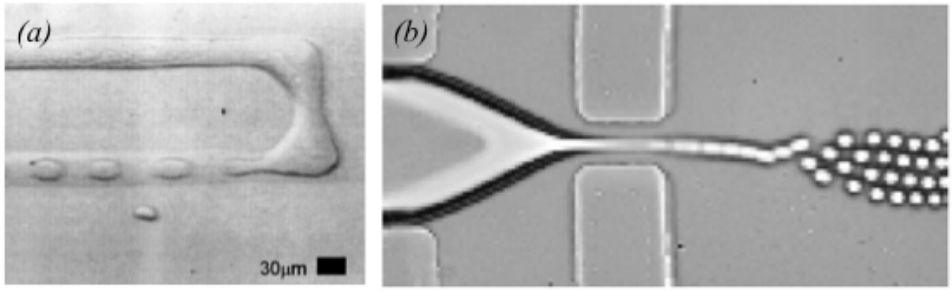


Figure 2.2 Microfluidic channel design competitive with Capillary instabilities (a) disperse phase on the top flow is introduced into continuous phase moving right to left at T-shape channel [42] (2) a stream of water flows between streams of oil and is geometrically focused into a narrow orifice. The jet is destabilized by the Rayleigh-plateau instability and forms small, monodisperse droplets. [37]

2.2 Droplet-based Microfluidics

Since the concept of miniaturization over traditional chemical progresses was given, recent studies have been dedicated on the development of droplet-based microfluidics as new methods in various fields of chemical, biological, and biomedical applications[42][43]. The demand of droplets as micro-reactors that manipulated picoliter volume for these biological and chemical analyses has been increased in the ability of lab-on-a-chip applications [44], thus offering novel platform of miniaturized system. Unlike the continuous flow systems, droplet-based microfluidics allows for independent control of individual droplet, which provides rapid mixing of reagents inside them [45], microparticles synthesis [46], microextraction [47], and protein crystallization [48].

These droplets can be fabricated with excellent control over the size (distributions), morphology [49], and composition by using immiscible two-phase system. It is hypothesized that the pinch-off droplets are formed by the imbalance between two immiscible fluids when they meet at junction are. The driving force related between action on deformation of the interface by viscous shear stresses and reaction on capillary pressure to resist the deformation would affect increase of interfacial instabilities (Ca <1). Capillary number ranges between 10^{-3} and 10^{0} in most microfluidic droplet-based devices [50]. Even though meaningful approaches have been attributed to experimental and numerical results, it has symbiotically faced the quantitative prediction of droplet regimes and droplet sizes as a significant task.

It is known that microfluidic structure plays a pivotal role in generating monodisperse droplets and flow rates [51]. Hence, the droplet's volume of from picoliter to nanoliter in water/organic system is determined by depending on Capillary number.

When Capillary number is low, the interfacial tension force dominates and viscous force and flow rate influence droplet breakup. Furthermore, multiple emulsion, Janus particles [54][55], porous particles [56][57], and core-shell droplet[58] are derived from difference droplet generating methods, and it leads to facilitate the acquisition of large amount of data. Okushima and Nisisako, et al [52][53] achieved double emulsions through two-step T-junction channel as shown in figure 3. Abate, A. et al [58] presented a simple system to from high-order multiple emulsions using multiple hydrodynamic flow-focusing. Shepherd, F. R. et al [54] and Prasad, N. et al [55] created Janus particles with distinct different compositions representing in Figure 5.

The main features of droplet-based microfluidics are generation and manipulation of droplets. Uniformity and size controllable ability are the most important in droplet generation methods. The aim of droplet formation is to reveal the regime of droplet generation field. Numerous techniques have been developed to obtain homogeneous droplets [50]. Uniformity of droplet size can be proved through the distribution of standard deviation of 1~3% [59][60]. Hence, monodispersity and uniformity for droplet generation in microfluidic device are highly required to confirm that micro-droplet produce constant, controlled and predictable behavior. Because of the economy of small volumes and ability for high numbers, droplet generation in microfluidic channel will have to be versatile and flexible so that micro-droplet as single reactor can detect oncogenes or other important disease genes in a high-throughput screening.

Therefore, since the perception of miniaturization became significant for biological and chemical sciences, water-in-oil (w/o) monodisperse particles have brought advantages of a lower cost and simple experimental format for high-throughput screening.

However, importantly, the field of droplet microfluidics so far relied on the use of organic solvents in combination with aqueous solutions to form droplets. A disadvantage of the use of organic solution is that they harm biomaterial upon encapsulation. The obvious solution is to include immiscible aqueous solutions also known as Aqueous Two-Phase Systems (ATPS).

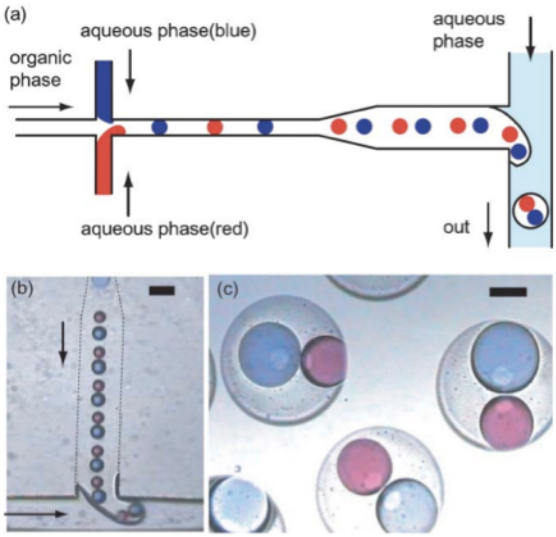


Figure 2.3 (a) Schematic diagram of Formation of double emulsions. (b) Aqueous droplets surrounding organic droplets at a hydrophobic T-junction. (c) Organic droplets surrounding blue and red aqueous drops. The diameter of the extranal droplet 175 µm [52][53]

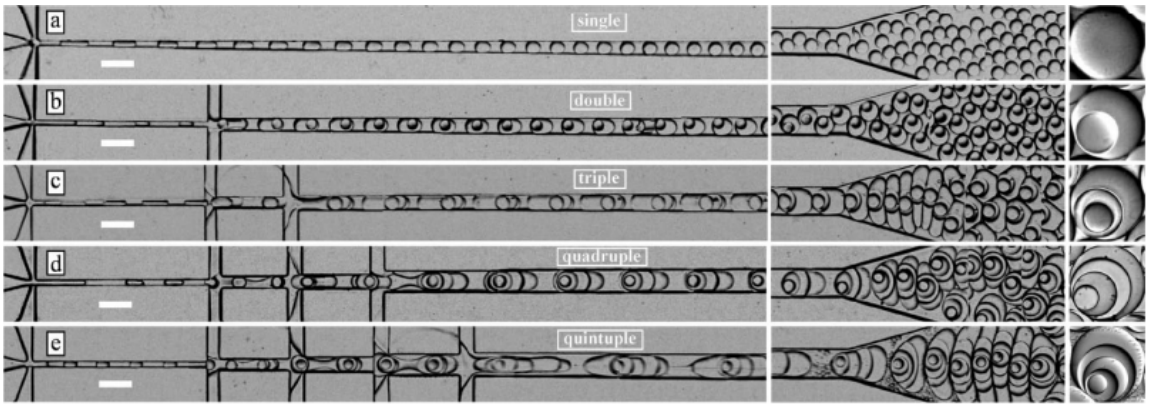


Figure 2.4 Drop maker arrays used to produce multiple emulsions with controlled order. Photomicrographs of a) single, b) double, c) triple, d) quadruple, and e) quintuple emulsion drop mark arrays. The multiple emulsions produced by the arrays are shown to the right [28]

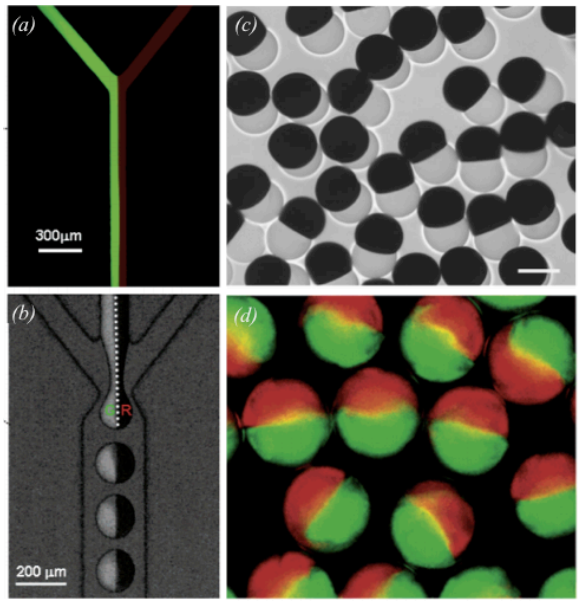


Figure 2.5 (a) Fluorescent image of Y-junction formed for production of Janus spheres (b) backlit fluorescence image (green excitation) illustrating that the FITC-silica microspheres remain sequestered in the left hemisphere of each granule generated. (c) Optical image of produced HJM with dumbbell shapes. (d) Fluorescence images of homogeneous spherical Janus particles. [54][55]

2.2.1 Droplet Formation

As discussed above, droplet formation is explained by the understanding of dimensionless number of capillary number, *Ca*, which is ratio of the viscous force to the interfacial tension. The droplet size distribution is in various water/oil system derived from the imbalance of interface between two phases. In the microfluidic systems, destabilizing the interface among solutions generates micro-droplets. Disperse phase stabilizes interface to promote the formation of jet applied by shear stress and fluid inertia [61]. Microfluidic methods for forming droplets can be either passive or active. Most methods are passive, relying on the flow field to deform the interface and promote the natural growth of instability [62][63]. Various size microstructure impacts on droplet generation depends immiscible fluids properties such as viscosity, interfacial tension, wettability to the material surface and other electric properties. A flow rate of the

dispersed phase and continuous phase flows, and its ratio is the factors that can be coordinated during the droplet formation procedure.

The two most common strategies are T-junction and Flow-focusing. These common techniques that are often used for generation of droplet in microfluidic system are dispersing fluid in a continuous phase with the configuration of co-flowing stream, cross-flowing in T-junction and flow-focusing. The dispersed phase and continuous phase meet at 90 degrees in a T-shape junction and the dispersed phase is squeezed by two focusing flow, imposing phase to pinch off illustrated in Figure 6. Depending mainly on the geometry of the microchannels, volumetric flow rate combination and relative viscosity of the fluids, the disperse phase is elongated and eventually broken into droplets of small volume [64]. These techniques are feasible particularly for fast generation of droplet of oil/water two-phase system with uniform size distribution.

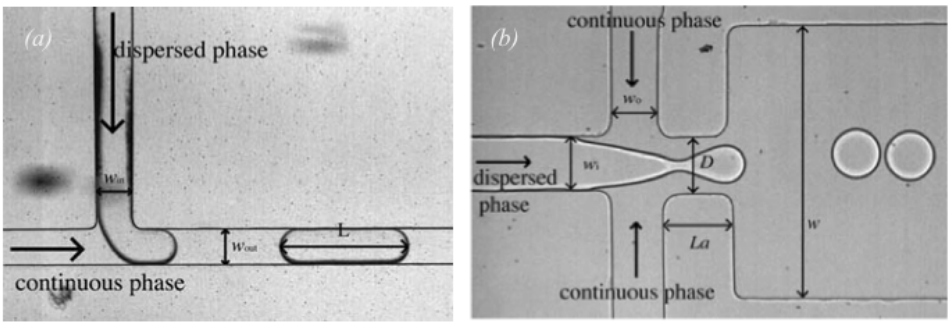


Figure 2.6 (a) Example of droplet formation in a T-junction. The dispersed phase and continuous phase meet at 90 degrees in a T-shaped junction (b) Example of droplet production in a flow-focusing device. The dispersed phase is squeezed by two counter-streaming flows of the carrier phase, forcing drops to detach [60]

2.2.2 Droplet-based Microfluidics Application

Application of droplet produced in microfluidic device has wide range from high-throughput biological operation to module integration over DNA amplification [44]. Multi-step microfluidic device should be prepared for sophisticated biological handling. Currently, therefore, integrated microfluidic device can be implemented for biological applications: (1) directed evolution – a yeast display library of an enzyme [65] (2) in vitro enzyme expression [66] (3) sensitive detection of cell-surface biomarkers on compartmentalized single cells [67] (4) toxicity screening [68]. For these reason mentioned above, droplet-based microfluidics behavior of transport, mixing, split, and sorting extended its application to therapeutic delivery, biomedical imaging, drug discovery, biomolecule synthesis, and diagnostics [10].

2.3 Aqueous Two Phase System

In the previous section I described microfluidic methods to produce droplets from oil and water system. In this section, I will discuss the production of water-in-water droplets through aqueous two-phase systems having more biocompatibility than water and oil system. We will firstly provide general background information on of ATPS build up from two or more immiscible aqueous polymer solutions in section 2.3.1 and then discuss the use of these systems in co-flowing microfluidic (section 2.3.2) and droplet microfluidics (2.3.3)

2.3.1 Introduction

Aqueous two-phase system (ATPS) consists of two immiscible water-based solutions of polymers or of one polymer and an inorganic salt, which can form phase partitioning. The formation of phase separation occurs due to pH, temperature and ionic strength of polymer solutions and concentration affects incompatibility of the polymers in the solution [69]. The most widely used ATPS is PEG/dextran system because Polyethylene glycol (PEG) conjugated biomolecules and can increase the solubility and stability of conjugated biomolecules in general [70][71]. A bottom phase mainly composes of dextran in systems, while a top phase mainly contains PEG. The phase separation is determined by binodal curve that represents the boundary separating from two-phase to single phase on a phase diagram shown in Figure 7. The phase diagram across binodal curve is essential for controlling ATPS microdroplets. ATPS droplets are emulsified in two-phase region and phases are not separated in single phases in terms of polymer concentration. Several factors influence aqueous two-phase system, such as molecular weight, concentration, pH, and etc [72][73]. The features ensure of APTS is a biocompatible environment but has a low value of interfacial tension in contrast to two phase systems comprised of water and an organic solvent. PEG/dextran phase separation commonly used for the extraction and purification of biomolecules [74-76]. ATPS have a high potential application in industry as a low cost tool, where ATPS containing polymers that are easily recycled are the most interesting for environment reasons [71].

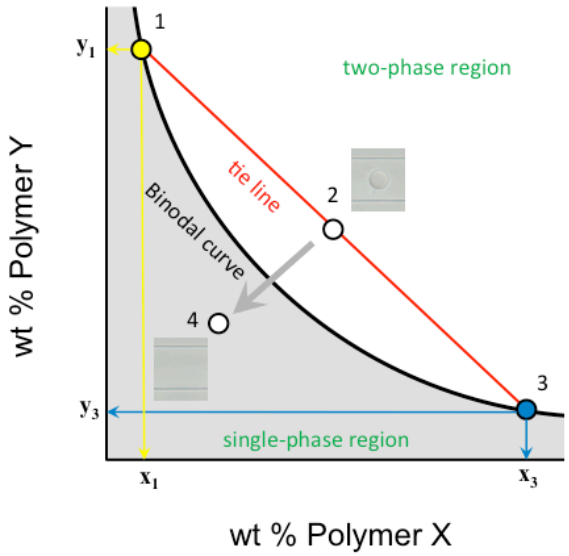


Figure 2.7 Determination of aqueous droplets containing phase-forming polymers defined binodal curve. The concentration of each polymer in the top (point 1) and bottom phase (point 3) is given by the intersection of the tie line

2.3.2 ATPS in co-flowing Microfluidics

Recently, ATPS have received increasing attention as a separation method in microfluidic device. Various molecules, including protein and cells have been successfully separated [77][78] because of the advantage of ATPS that no special methods are required to stabilize flows. Hu et al [77] implemented protein purification and extraction from Hela cell within microfluidic aqueous two-phase system shown in figure 8 (a). FITC labeled hydrophilic proteins migrated into PEG phase from hydrophobic detergent. Meagher et al [79] revealed that protein separation or purification is employed by diffusion between co-flowing methods as shown in figure 8 (b).

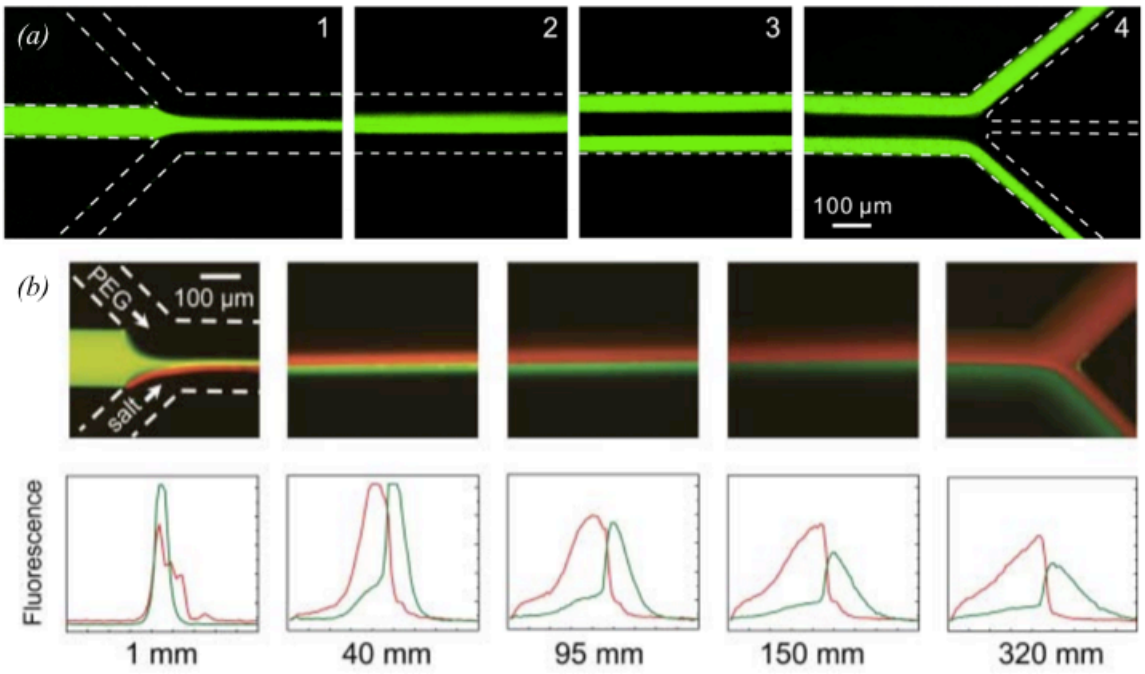


Figure 2.8 (a) Partitioning of fluorescently labeled molecules between three co-flowing streams in a microchannel (b) Fluorescence micrographs representing the sample partitioning in a microchannel

Moreover, other studies have demonstrated the separation and purification of membrane protein [77], and bacteriorhodopsin[80]. Cell separation including plant cell [81], animal cell [82], and human cell [83] in ATPS has been also rarely reported by several pioneer groups. However, applications on the cell separation are very limited that should be more investigated in the future.

2.3.3 ATPS in Droplet-based Microfluidics

Under the conditions of typical microfluidic channels, it is difficult to from droplets spontaneously when two aqueous polymer solutions meet at junction area. However, due to biologically friendly environment, water-based ATPS is attractive approaches for various biomaterials [17][84]. Vijayakumar et al [85] Double emulsions

of PEG and dextran are formed at T-junction within oil-based continuous phase for cell extraction. Figure 9 indicate droplet formation, water-in-water-in-oil droplets, and cell extraction. One approach relies on the use of a chaperoning oil stream to form the droplets. Besides of using oil solution, monodisperse water-in-water emulsion droplets are developed by several research methods [86-90]. In the potential view of previous studies for aqueous droplet's application, it is able to be to provide individual micro-environment in aqueous two-phases system (ATPS) will be able to a more effective solution for quantitative cell biology and enzyme/protein separation [91].

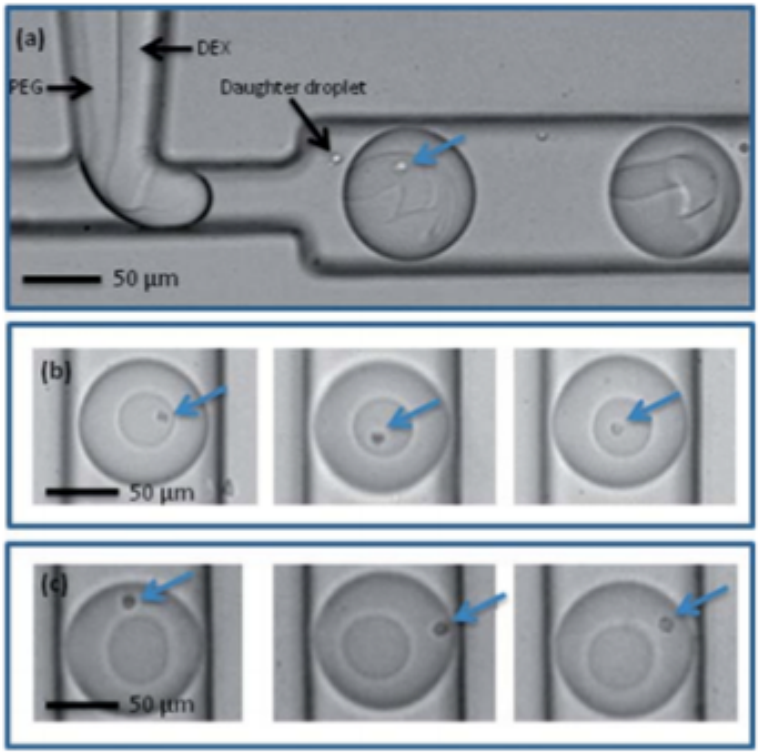


Figure 2.9 Cell with Ab-NIPAM within dextran at a microfluidic T-juntion (a) and in a dextran droplet prior to mixing (b). After mixing, cells partition to the enter PEG phase (c) [85].

2.4 Summary

Through the literature survey in this chapter, we explored the different techniques, methods and processes of droplet-based microfluidics and aqueous two-phase systems. The researches on water-in-oil (w/o) monodispersed particles made through droplet-based microfluidic device did not only lead to the achievement of uniformity and size-controllable particles but also allowed the use of these particles into diverse applications in biology and chemistry. As shown in this chapter, ATPS in droplet-based microfluidics could be a powerful tool to access water-in-water types of particles but it is challenging to handle its low interfacial tension in microfluidic device.

Therefore, the research in this thesis conducted with the purposes as below: (1) to demonstrate geometry effect on ATPS droplet formation, (2) to study the general limitations to break up droplets, (3) to determine suitable ATPS droplet formulation condition, (4) to establish a stable droplet size.

In this thesis, the effect of microfluidic device geometry on ATPS droplet generation has been surveyed. A typical process of droplet formation in microfluidic device with T-junction, cross-junction, and double cross junction will be described.

Chapter 3

Dextran Droplet Generation in Microfluidic ATPS

This chapter is about monodispersed microdroplet generation in microfluidic aqueous two-phase system (ATPS). Droplet generation using aqueous two phase systems (ATPS) in microfluidic device was studied by various junction areas which were considered as T-junction, flow-focusing, and double-flow-focusing (or H-junction). Disadvantage of low interfacial tension and high viscosity between aqueous phases make it experimentally difficult to produce uniform micro-droplets. A double-flow-focusing microfluidic device is able to be a crucial method to generate water-in-water (w/w) droplets due to the stability of dispersion between two junction areas. When second continuous phase was injected, the pressure between two junctions has been changed well controllable for droplet breakup. By comparing each of channels, such as T-junction and flow-focusing which are widely used in microfluidics, double-cross junction could prove that uniform ATPS droplets were generated. Various sized droplets were

also pinched off among T-junction, flow-focusing, and double-flow-focusing, representing the possibility of uniform droplets and the regions of droplet generation in ATPS. It was also estimated for the standard deviation of droplet size to demonstrate the uniformity in T-junction, flow-focusing, and double-flow-focusing, respectively.

3.1 Introduction

Aqueous two-phase systems (ATPS) consist of two immiscible fluids in a bulk water solvent [92]. High water content in such systems can give a mild condition for large scale separation of biological materials, allowing the stabilization of biomaterials, a first step of isolation, an additional preparative application, and analytical applications [93]. Since the study of droplet generation using ATPSs in macro scale [94], ATPS droplet formation has been recently advancing in microfluidic device and resulting diverse applications for cell extraction [82] and diffusive mass transfer [79]. ATPSs have extremely low interfacial tension (less than 10^{-4} - 10^{-6} μN/m) more than organic/water system (more than 1 - 100 mN/m) [95][96]. Therefore, it is necessary for controlling of brittle and elastic interfacial tension and high viscosity in microfluidic device. The low interfacial tension disturbs the driving force impact on droplet generation. Therefore, to handle of low interfacial tension of ATPSs in microfluidic device has become a major obstacle for droplet generation since the beginning of study of ATPS droplets [89][97].

Several research groups have solved this experimental challenge with difference approaches. However, all experimental results derived from external factors to cause perturbation of hydrodynamic focusing. The electrodes [34], multi-layers microchannel

with orifice combined with Braille pin valve [89], piezoelectric cantilevers [95], and mechanical vibrator [88], respectively, were embedded inside microfluidic device. To obtain monodispersity with the low interfacial tension in ATPSs, it is required to periodically change dispersed phase. In contrast to these external installations, double-flow-focusing channel drew high-throughput screening advantageous of simple method, cost effective, and heavy workload. Alternatively to the continuous mechanical pressure by pressure-driven flow, no external actuations were used.

Hence, in this chapter, we demonstrated the effect of double-flow-focusing channel to produce water-in-water (w/w) droplets in microfluidic device. T-junctions and flow-focusing, broadly used for microfluidic device, were compared with double-flow-focusing channel. This approach consists of a series of two flow-focusing junctions where one dextran phase (dispersed) and two PEG phases (continuous) were injected into the device. Intriguingly, theses droplets can be developed within a combination range of flow rate conditions where the droplet regime can be predicted. Thus, the double-flow-focusing microfluidic device brings the possibility of uniform water-in-water (w/w) droplets with an extremely low interfacial tension of ATPS.

3.2 Materials and Methods

For aqueous two-phase systems, Poly(ethylene glycol) (PEG MW 8000 and 20000, Sigma-Aldrich Co., St. Louis, MO, USA) and dextran (MW 500000 and 40000, Sigma-Aldrich Co., St. Louis, MO, USA) were used as immiscible aqueous phases in microfluidic device. The feature of ATPS that purple dye was in water phase and not in oil phase, while PEG contained most dye and some as consisted within dextran phase

represented in figure 1. PEG and dextran were dissolved into deionized water and Phosphate buffered saline (PBS) (10x, Sigma-Aldrich Co., St. Louis, MO, USA) to be 20 w/w % PEG and 10 w/w % Dextran, respectively. These molecular weights were chosen for the potential of biological application in the future. The physical properties of the dextran-rich and PEG-rich were obtained from published data for ATPSs [98][99].

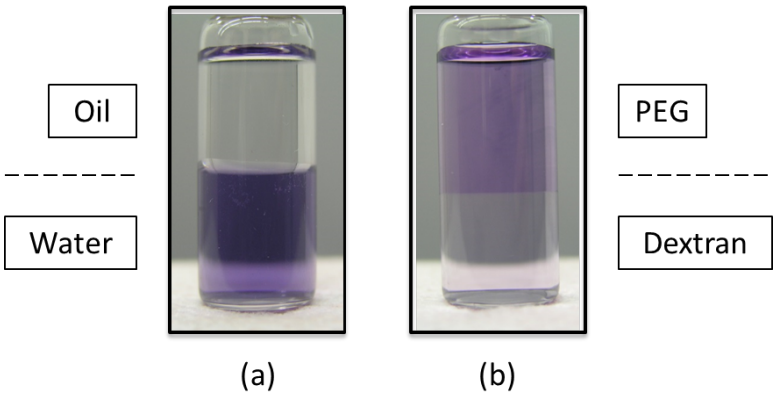


Figure 3.1 (a) water and oil system (b) aqueous two-phase system (ATPS) in macroscope, respectively. Unlike water/oil system, ATPS facilitates mass transfer through interface effectively.

3.3 Fabrication of Microfluidic Device

Microscale complex structures devices and systems (1μm - 100μm) can be manufactured through micro-electromechanical system (MEMS) technology. The major advantages of MEMS are that they are smaller, lighter, faster, and usually more precise than their macroscopic counterparts. The development of MEMS devices requires specific fabrication technologies, usually involving a structured sequence of operations that enable precision, flexible design, control systems manipulation, repeatability, and accuracy [100]. From the processing of photolithography and soft-lithography (or PDMS replica processing), microfluidic device was prudently fabricated. (Figure 2) The photolithography technique was used for making a microchannel mold on a silicon

substrate (SU-8 50, MicroChem Corp., MA, USA) resulting from spin coat, soft-baked, exposure to UV light through a chrome mask, and Post-Exposure-Baked. The height of mold was 150μm. The water is rinsed in a developing solution (SU-8 developer, MicroChem Corp., MA, USA) which removes the unexposed area of photoresist, and exposed areas of photoresist in case of negative photoresist leaves micro-patterns illustrated in figure 2 (left).

After development, a microfluidic device was prepared with which Polydimethylsiloxane (PDMS) (Dow Corning Sylgard 184) was mixed with curing agent at 10:1 ratio, poured onto the microchannel mold, cured at room temperature for 2 days, peeled from the mold, cut to desired size and punched with holes at the end of the channels for tubing connection with a tip diameter of 0.75 mm (Harris Uni-Core Co., USA). The surface of the master mold was functionalized with trichloro(3,3,3-trifluoropropyl) silane (Sigma-Aldrich, USA) for stripping the hardened PDMS replica easily after molding show in figure 2 (right).

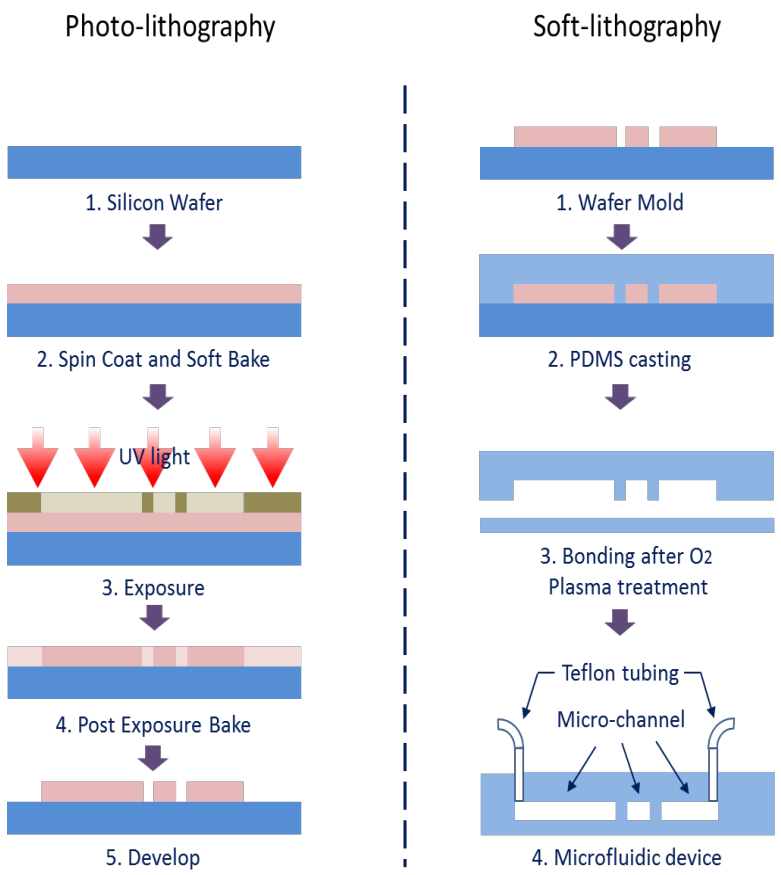
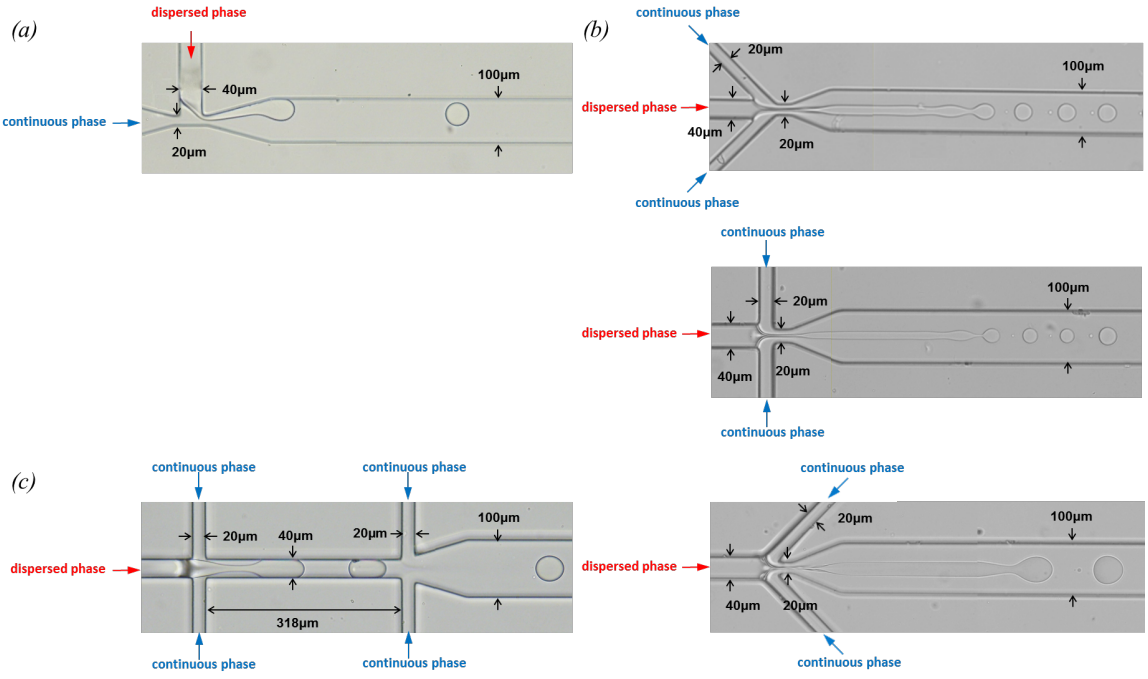


Figure 3.2 Schematic of fabrication process for microfluidic device *via* photo-lithography (Left) and soft-lithography technologies (Right)

3.4 Experimental Setup

The monodispersed water-in-water emulsions were prepared by utilizing pressure-driven-based microfluidic device. The round Polytetrafluoroethylene tubing (OD 0.016 inch, ID 0.004 inch, Cole-Parmer Co., USA) was inserted inside microchannel. For formation of ATPSs droplets, PEG as continuous phase and dextran as dispersed phase were injected into microfluidic device connected with pressure pump (Mitos P-Pump, Dolomite Microfluidic Co., Royston, United Kingdom). Three pressure-driven pumps were conducted for T-junction, Flow-focosing, and double-cross junction microfluidic

device. All the operation of pump and microscope were connected and controlled by using computer software program provided by Dolomite Microfluidics Company. For the microfluidic approach, PDMS microfluidic device was observed through an inverted microscope (Olympus BX53F, Tokyo, Japan) equipped with a charge-coupled device (CCD) colour camera (Olympus DP72, Tokyo, Japan). The 20x objective lens was used, and the exposure time was set to 100 ms with ISO 800 to take images (resolution 1360 x 1204 pixels, 15 fps), respectively. The dimension of microchannel, experimental setup, and microfluidic device injected through tube are shown in figure 3. At least more than 200 ATPSs droplets were collected and measured for diameter of droplets. The diameter of droplets was measured by using an open-source image processing software, Image J after captured through CCD camera. A frame of pictures was captured after the liquid broken into a drop threaded through the focusing nozzle. A typical set of flow rate by pressure-driven pump for the dispersed and continuous phases was 50 mbar and 300 mbar, respectively.



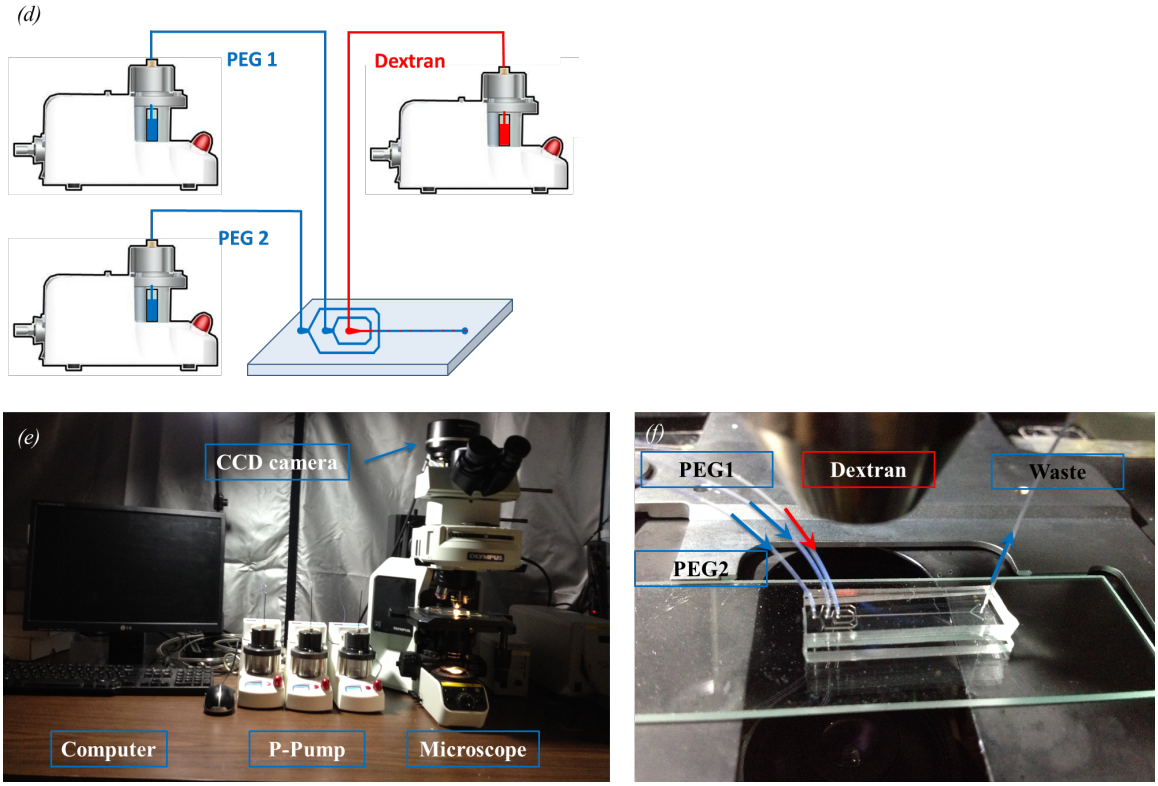


Figure 3.3 Dimension of ATPS droplet-based microfluidics with (a) T-junction (b) flow-focusing in 45, 90, and 135 angles, (c) double-flow-focusing, and (d) pressure-driven flow conducted with microfluidic device, (e) Experimental setup (f) ATPS microfluidic device.

3.5 Droplet Generation in T-junction

A typical process of formation of droplets in the T-junction can be studied as follows. When ATPS solutions are injected, the two aqueous fluids form an interface and droplets through main channel. Here is an observation that dispersed phase, dextran flow, flows along the channel wall without breaking off in T-junction. The size of the droplets relies on the flow rates of the two solutions. Increased continuous phase led to small diameter of droplet with ratio of dextran pressure to PEG pressure. The fact that the shear force affect interface between the two fluids driving the generation of droplets is well known. However, unlike previous studies that there are two regimes revealed as the

parameters are varied: dripping, and squeezing [63], the dispersed phase (Dextran), overall, shows the characteristic movement sticky to the wall in all range of flow rate s. It can be discussed that when fluid meet at junction, the dispersed phase are much stable and adhesive to the wall, thus it is difficult to break-off for the formation of droplets. Also, even though the rate of the continuous phase increases to impact on droplet pinch-off, shear force cannot overcome the interfacial tension illustrated in figure 4. The arbitrarily generated droplets and various sizes of droplets also cannot be controlled by the flow rate representing in figure 5. The result of T-junction presents ATPS characteristics that dextran stream met PEG at junction pinch off in terms of pressure change. However, the uniform droplets could not be generated because of low interfacial tension of ATPS characteristic and high adhesion to PDMS. The droplets were generated not in junction area but in main channel, categorizing into squeezing regime only. For transition from squeezing to dripping in T-junction, the capillary number should be high enough so that spherical droplets can be emitted [63]. On the contrary, behavior of two aqueous fluids in T-junction in contradiction to two immiscible ones does not match to previous studied. Therefore, the adhesion of dispersed phase overwhelms any other factors, such as shear force and interfacial stresses.

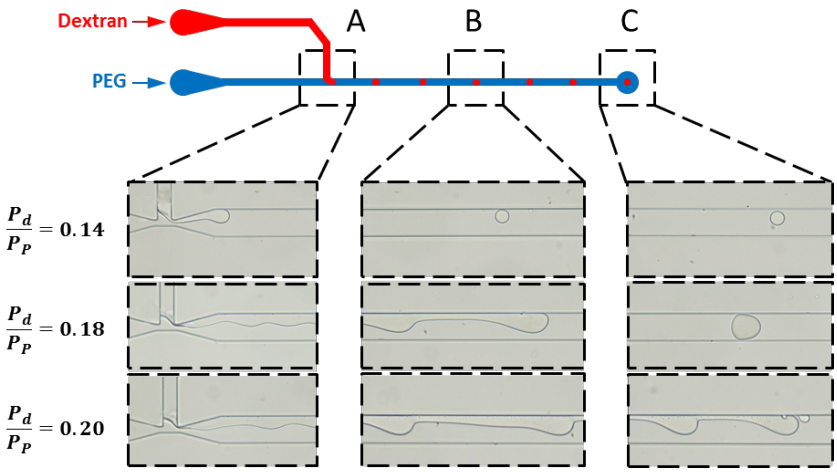


Figure 3.4 Droplets produced using pressure controlled setup in T-junction.

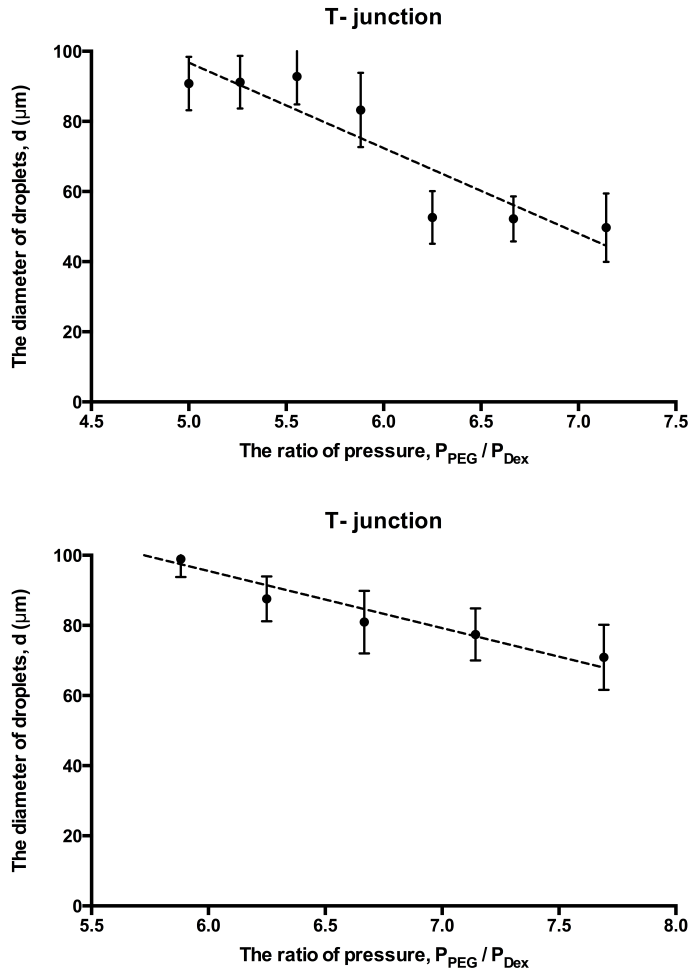


Figure 3.5 Varying PEG/dextran pressure ratio changes the diameter of spherial droplet in T-shape microchannel. (Top) PEG(MW 8000, 20%, w/v(weight per volume)) and Dextran (MW 500000, 10%, w/v), (Bottom) PEG(MW 20000, 20%, w/v) and Dextran (MW 40000, 10%, w/v), respectively.

3.6 Droplet Generation in Flow-focusing with Different Angles

Common design for micro-droplet generation, called flow-focusing, is investigated on ATPS droplet formation. As water-in-oil droplet is made, dextran stream as dispersed phase is breaking off in cross-junction. A typical example of a flow-focusing is described that continuous phases perpendicularly meet. The dispersed phase is squeeze by two counter-flowing streams of the continuous phase. Generally, droplets can be formed in either dripping or jetting regimes. Flow rate, viscosity ratio of both phases, and channel geometries as factors of droplet breakup dynamics affect the droplet generation [63]. Transition from dripping to jetting resulted in Rayleigh-Plateau instability and the growth time of the dispersed thread [101]. However, dextran droplet's size has a variety of diameters in flow-focusing and the total flow rate influence the size of droplets. Dispersed phase did not break up at focusing area and keep jetting flow. Controlling the flow rate can reduce the thread size and distance, but the one in flow-focusing does break up irregularly. The deviation of droplet size in various range of flow rate proves that droplets are formed randomly. To obtain a better understand of shear stresses impact on fluid instabilities, three different types of flow-focusing channels are prepared: 45, 90, and 135 degree shown in figure 6. As a result of fluid regime in different flow-focusing, 135 degree flow-focusing applies more force to dispersed phase and leads to reduce the deviation value compared to 45 and 90 degree. However, viscous forces did not increase the growth of deformation of thread to pinch off. From the view of inertial force, elongation of the dispersed phase cumulated and kept the formation of long fluid thread. Hence, the uniformity of droplets was not demonstrated in flow-focusing. It is extremely difficult for disperse stream to transfer to dripping regime.

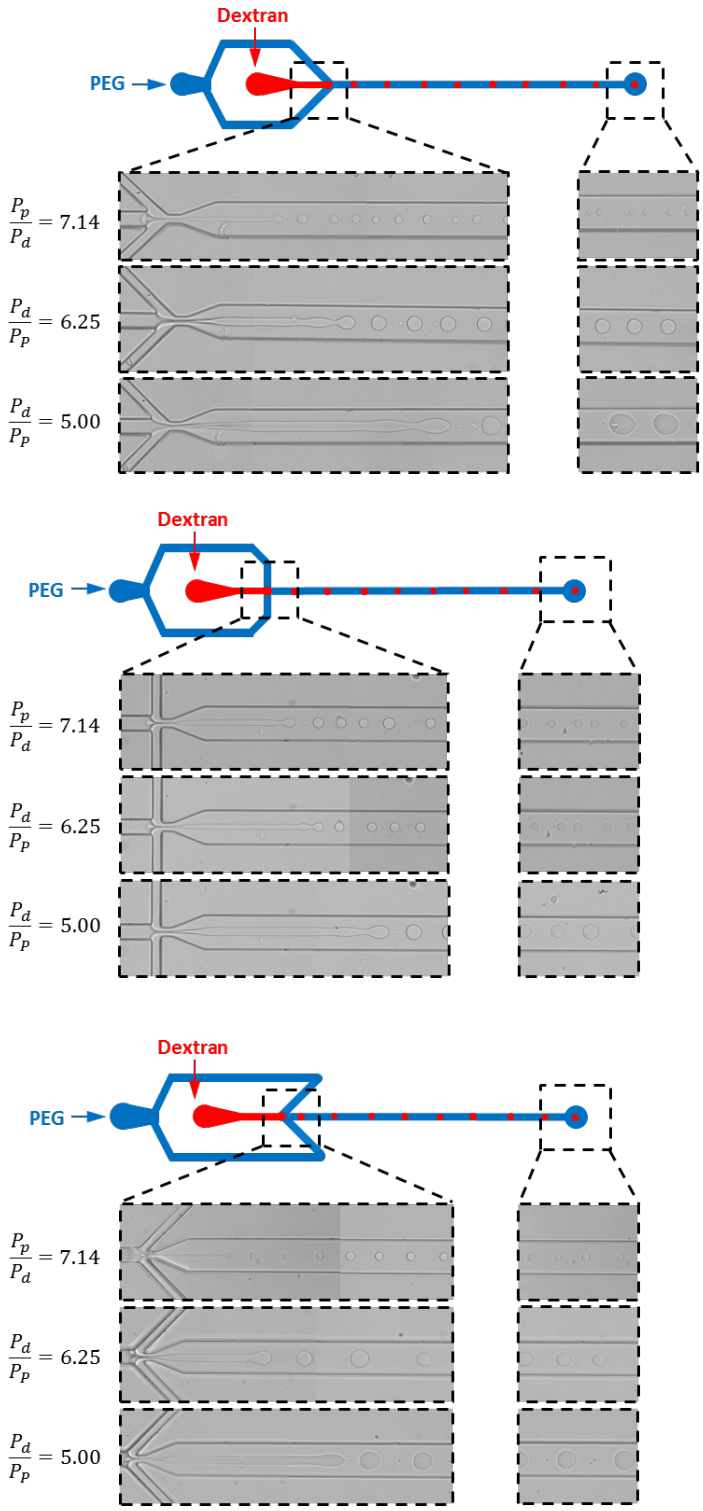


Figure 3.6 Droplets produced using pressure controlled setup in terms of pressure ratio. 60°, 90°, and 135°. Two continuous phases focus disperse phase and break it into polydispersed droplets.

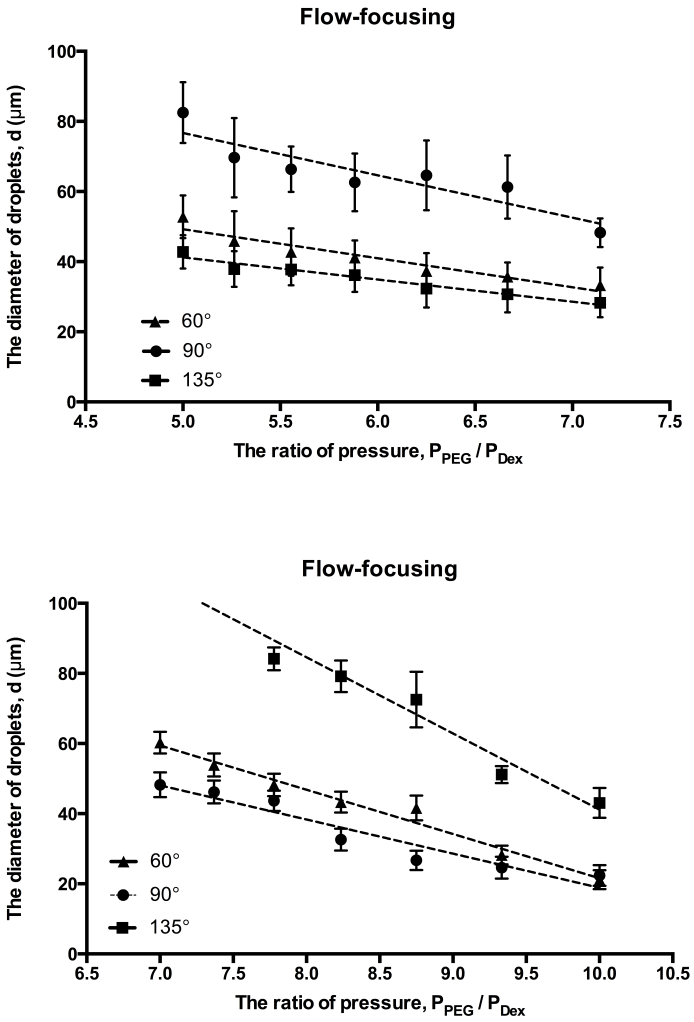


Figure 3.7 Varying PEG/dextran pressure ratio changes the diameter of spherial droplet in flow-focusing microchannel. (Top) PEG(Mw 8000, 20%, w/v) and Dextran (MW 500000, 10%, w/v), (Bottom) PEG(MW 20000, 205, w/v) and Dextran (Mw 40000, 10%, w/v), respectively.

3.7 Droplet Generation in Double-flow-focusing

In this work, we used a simple method added one more crossed junction next to the former crossed junction for stable fluid dynamics. With double-cross junction in microfluidic device, we drew a visible conclusion on the effect of geometry, concentration, and various pressure-controlled flows conditions for the hydrodynamics of

breakup of two immiscible fluids. Double-cross junction device consists of a series of two flow-focusing which one stream of dextran as disperse phase and two stream of PEG as continuous phase were injected. In conclusion, double-cross junction area could lead to well-controlled ATPS droplet break up. Previous studies of junction geometry for droplet generation in microfluidic device implemented only attention of channel geometry; 1) upstream, dispersed and continuous flow channel width, and 2) downstream, narrow orifice [51][102]. Furthermore, transition between jetting and dripping induced by increasing or decreasing flow rate regarding capillary number [63]. The main observation in cross junction area presented that various sizes of droplets were formed with changes by pressure-driven pump. Additional flow-focusing make it possible to generate monodispere w/w droplets as shown in figure 8. However, due to the characteristic of ATPS stream stretching with one cross junction, additional installation should be conducted regardless of those factors above. It was observed that the dispersed phase had stretchiness because of biocompatible polymer solution. Therefore, flow-focusing device with double-cross junction was a valid method for fine ATPS droplet break-up.

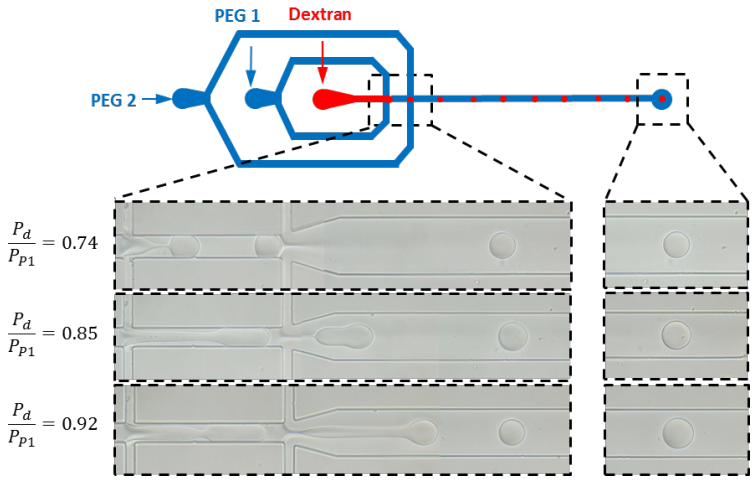
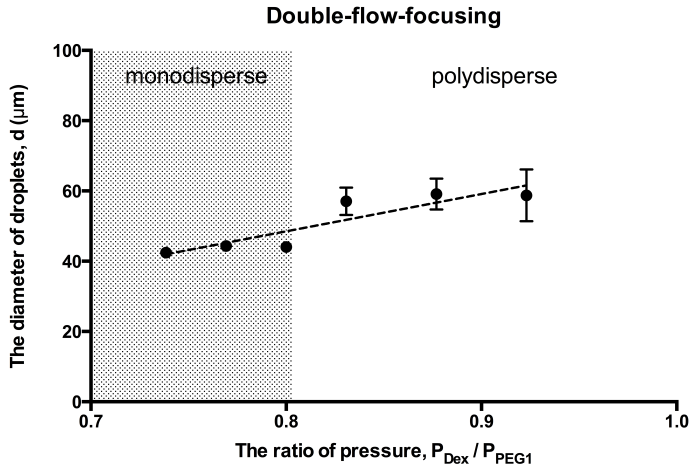


Figure 3.8 Formation of microdroplets using double-flow-focusing and droplet generated between two junction areas due to constant pressure enhanced in terms of pressure ratio.

3.7.1 The Change of Flow Rate of Dispersed Phase: Breaking-up Location

Various sized droplets in terms of dextran phase change were generated between two cross junctions. Each droplet size had 2% of deviation away from the average droplet size in monodispersity area. The higher is flow rate of dextran, the more increase deviation value of droplet size. As fluid transfer region from monodispersity to polydispersisty in terms of flow rate of dextran phase, disperse phase loses their uniformity breaking through the line of demarcation of second junction are in figure 9. Pressure distribution between two junctions area make it stable for ATPS droplet generation. Moreover, the breakup location relies on the change of flow rate of disperse phase. The disperse phase has a forward movement up to second junction when increased. When flow rate of dextran continuously increases, distance of breakup moves forward downstream which the average size of droplets was barely deviated.



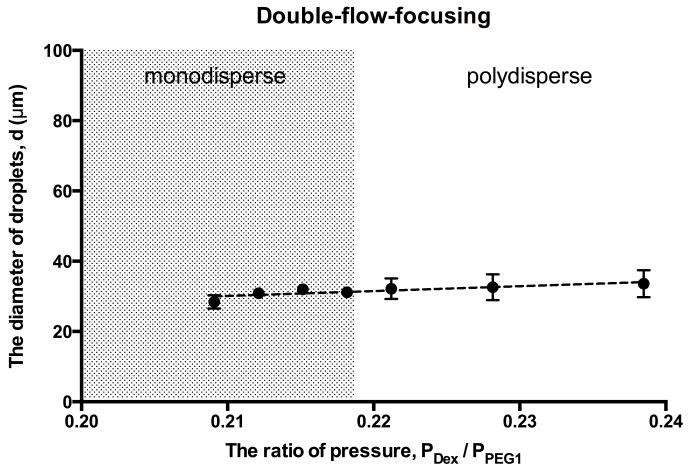


Figure 3.9 Dextran change impacted on monodispersity and polydispersity in PEG (Mw 8000, 20%, w/v) and Dex(MW 500000, 10%, w/v) (top), and PEG(MW 20000, 20%, w/v) and Dextran(MW 40000, 10%, w/v) (botton), resprectively. Dextran flow impact on droplet generation moved the location of pick off of disperse phase, which lead to polydisperse region

3.7.2 The Change of Flow Rate of First Continuous Phase: Droplet Size Control

Droplet size formed regularly between two cross junctions, but the value of standard deviation where droplet generated close to second junction was larger than the one where droplet generated close to first junction. The effect of first PEG flow rate influenced to disperse dextran size. In pressure distribution between focusing junctions, first continuous phase afford droplet size diversity as one in typical organic/water system [103]. However, dispersed flow was beginning to be loosely stretched before they reached second junction area. This is interpreted that thinner thread of jetting flow breaks up earlier and became small droplet size due to strong viscoelastic force. The

characteristic of polymer solution such as ATPS is that fluids have non-Newtonian and their dynamics of viscoelasticity play a vital role in droplet formation in ATPS. In this perspective, first junction area is able to regulate the interface force between two aqueous phases within suitable pressure distribution dawn by second junction are. When PEG1 flow rate increased, the size of droplets are decreased and transition from jetting to dripping is observed in terms of first continuous phase rates presenting in figure 10.

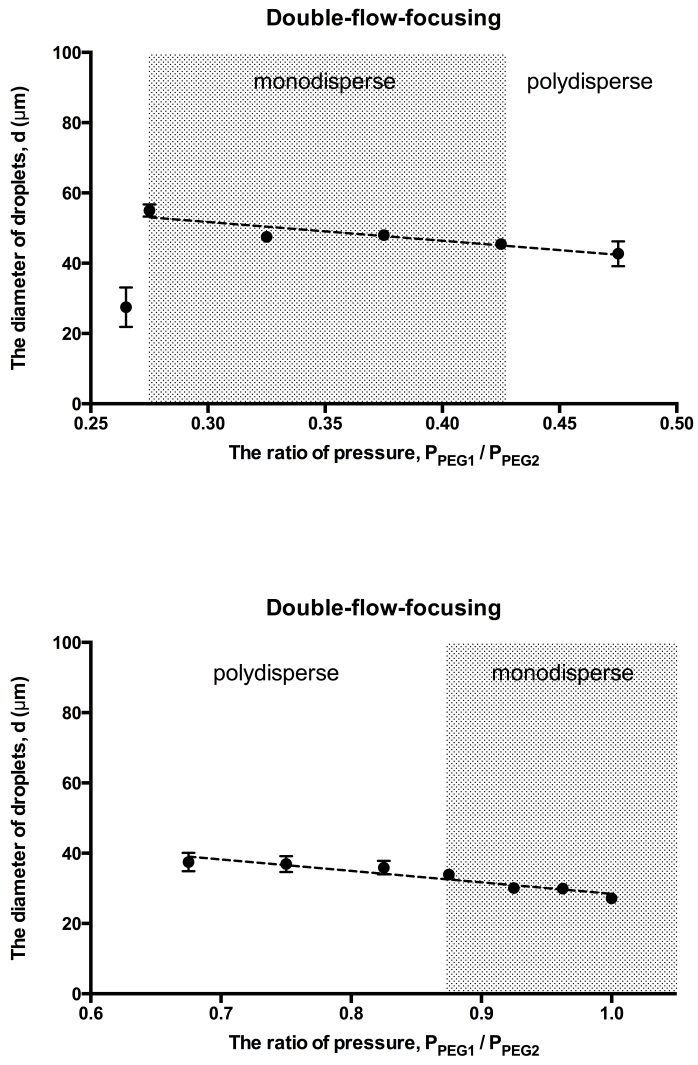


Figure 3.10 PEG1 change impacted on monodispersity and polydispersity in PEG (MW 8000, 20%, w/v) and Dex(MW 500000, 10%, w/v) (top), and PEG(MW 20000, 20%, w/v) and Dextran(MW 40 000, 10%, w/v) (bottom), respectively. Within monodispersity area, droplet size can be controlled in terms of PEG1 flow rate.

3.7.3 The Change of Flow Rate of Second Continuous Phase: Distance Between Droplets

Dextran droplet regularly generated in high flow rate of second PEG. The deviation of droplet size was less than 2% away from the average size, while various droplet sizes were formed in low flow rate of PEG2 in second junction. Hence, the effect of second cross junction has played an essential role in uniform droplet generation.

Even though flow rate of PEG2 in second junction increased and decreased less than dextran flow rate, droplet size were not changed but uniformly generated. We concluded that there is no size impact on the change of flow rate of second PEG phase within the monodispersity area in figure 11.When second PEG flow rate came down to polydisperse boundary, dextran was adhered to the wall and continued to move out of second junction and then, various droplet sizes were formed. It is crucial to discover certain flow rate of PEG2 to make it stable for monodisperse droplets with dextran and PEG1 flow rates.

Average of droplet sizes in terms of difference of second flow rate of PEG was not dramatically changed, but standard deviation was decreased by increasing the PEG flow rate so that dextran droplet could be formed regularly in downstream channel. Higher second PEG flow rate, longer distance of between droplets in microchannel that did not impact on droplet size. We conclude that transition from jetting to dripping make is possible with additional junction changing pressure distribution. Distinctive feature of viscoelasticity reaches a conclusion that for ATPS fluids, the escalation tension in the thread has small influence on the capillary number. The size of droplet generated in terms

of PEG2 is not impressively controlled, but PEG2 phase present the distance between droplets when it increases shown in figure 12.

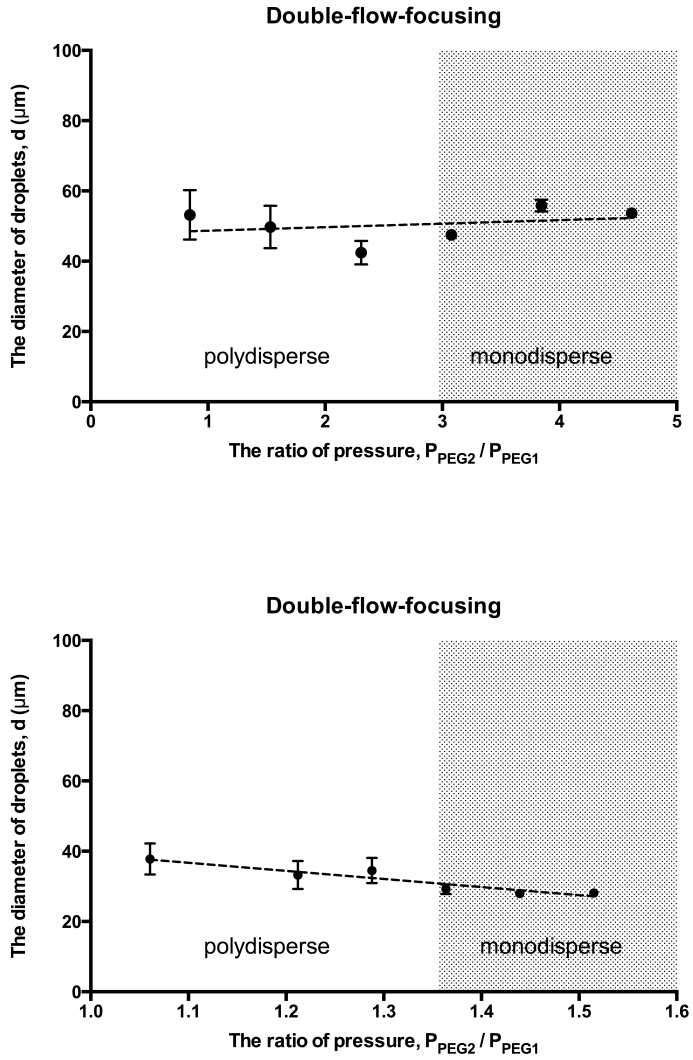


Figure 3.11 PEG2 change impacted on monodispersity and polydispersity in PEG (MW 8000, 20%, w/v) and Dex(MW 500000, 10%, w/v) (top), and PEG(MW 20000, 20%, w/v) and Dextran(MW40000, 10%, w/v) (bottom), respectively.

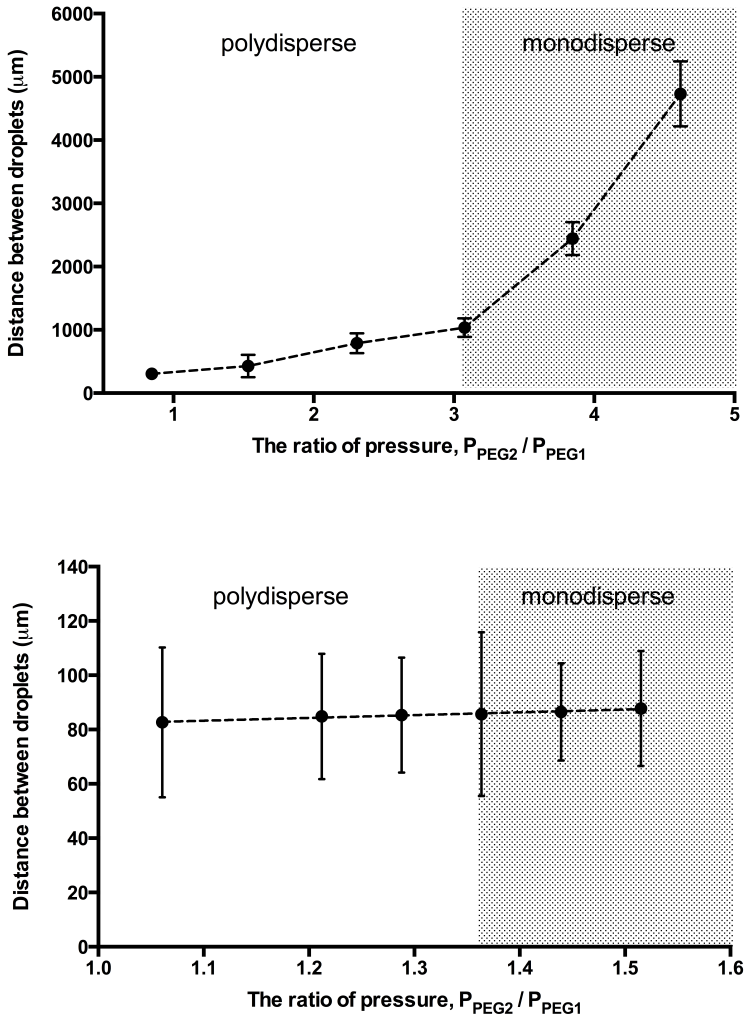


Figure 3.12 Distance between droplets in terms of PEG2 flow rate over PEG1 flow rate. PEG (MW 8000, 20%, w/v) and Dex(MW 500000, 10%, w/v) (top), and PEG(MW 20000, 20%, w/v) and Dextran(MW40000, 10%, w/v) (bottom), respectively. Effect of PEG2 is highly acting on increasing the space between droplets

3.7.4 Stable and Unstable Regime

Dextran droplets uniformly generated in terms of pressure-driven flow rate. As dextran increased with fixed PEG1 and PEG2 pressure, stable and unstable region can be existed at certain range of dextran pressure ratio. The average droplet diameter is 47um. Dextran droplet regularly generated with PEG pressure changing. The uniform droplets

generated with PEG1 changing and the stable region was considered getting increasing into stable region. Droplet size did not changed at stable region in term of PEG2 pressure. In unstable region, which is outside slope, highly decreased up to certain PEG2 pressure in stable region and uniform droplet could be dispersed. Various droplet sizes were formed in low pressure of PEG2 in second junction. When PEG2 pressure was smaller than PEG1 pressure at fixed dextran pressure, droplet did not uniformly generated and focus-flow moved out of second junction. To obtain stable droplet size, PEG2 pressure at least larger than PEG1 pressure so that droplet could be formed between double cross junctions. High concentration of polymer solution involves the increase of viscosity that more viscous force applied to disperse phase to pinch off. Two types of concentration of ATPS having different interfacial tension were demonstrated. It is revealed that 25 wt% PEG expected larger interface force than 20 wt% is more loaded by PEG1 and PEG2 flow rates in figure 13.

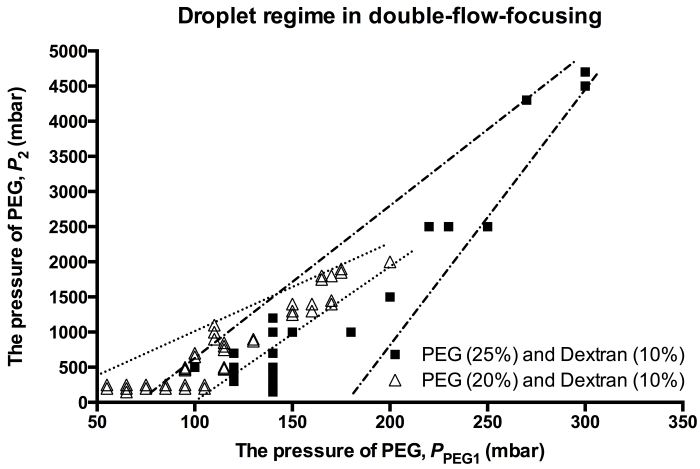


Figure 3.13 Range of droplet generation in terms of molecular difference

3.8 Conclusions

In this work, we demonstrated water-in-water droplet generation in microfluidic device using aqueous two-phase system. Due to extremely low interfacial tension, additional flow-focused channel design would be alternative and new approach to solve the difficulty of droplet generation. Through the combination of a disperse phase and two continuous phases in double-flow-focusing, we are able to generate droplet size with diameter less than 100 μm. The additional flow-focusing junction in double-flow-focusing design creates uniform and controllable size microdroplets. Double-flow-focusing microchannel provides a versatile feature in designing ATPS droplet-based microfluidic device. Therefore, we expect that the information of uniform ATPS droplets regime provide more biological and chemical applications [22][23]. There are various cases of ATPS using polymer-polymer or polymer-salt in terms of molecular weight and concentration. Each of one is widely used for certain biological applications. Hence, size controllable and uniform droplets in terms of different types of aqueous two-phase system should be investigated in the future.

Chapter 4

Poly(ethylene glycol) (PEG) Droplet Generation in Microfluidic ATPS

This chapter will examine Poly(ethylene glycol) PEG monodispersity in double-flow-focusing design within microfluidic device. Because of low interfacial tension and depending on phase partitioning condition, PEG droplet generation has restrictions in droplet generation. As we investigated dextran droplet generation within double-flow-focusing, it is essential for both dextran and PEG droplet to expand the possibility of double-flow-focusing. In section 4.1, a PEG as disperse phase, and two dextran, as continuous phase, will be prepared to conduct PEG droplet generation. The following section (4.2) will extends previous work on droplet generation in aqueous two-phase system microfluidics to interfacial tension effects constructed by the presence of surfactants.

4.1 Introduction

An aqueous two-phase system (ATPS) droplet-based microfluidic approach has been implemented by double-flow-focusing to generate aqueous emulsion and jets. Due to the advantage of biocompatibility, monodisperse water-in-water emulsion can be used as platform for biomaterials [104]. Extremely low interfacial tension by the characteristic of ATPS leads new strategies to manipulate fluid flows. In this section, as we conducted above for dextran droplet generation through double-flow-focusing, PEG emulsion within dextran phase has been investigated. The PEG emulsion will be able to take account of biocompatibility and eco-friendly surroundings in biomedical applications [78][105-107]. Common solutes for aqueous two-phase system that two phases separated into water above the critical concentrations are summarized in Table 4.1. The two liquid phases in ATPS are formed from the uneven distribution of the components. At low concentrations of polymer, the solution exists as a single phase, and at high concentrations phase separation occurs. A binodal curve separates these two regions of the diagram and then, compositions for each phase of a phase separated ATPS lie on this curve. The condition of two phases or single-phase separation exists at micro scale and should deal with droplet formation in aqueous two-phase system microfluidic.

Table 2 List of common aqeuous two-phase systems formed with biocompatible additives. In each column, solute A and solute B represent a pair of incompatible solutes. In addition, some proteins and polysaccharides, such as sodium caseinate and sodium alginate can also form a biocompatible ATPS [105].

Solute A	Poly(ethylene) glycol (PEG	Dextran	Methylcellulose
Solute B	Dextran	PEG	Dextran
	Polyvinyl alcohol (PVA)	PVA	PVA
	Polyvinyl pyrrolidone (PVP)	PVP	PVP
	Tripotassium phosphate (K_3PO_4) Sodium citrate or Sodium sulfate	Methycellulose	Na_2HPO_4

4.2 Droplet Generation in Double-flow-focusing

Manipulation of PEG jetting flow in microfluidic device has become an indispensable method for separation of DNA [108], proteins [79], and cells [182]. Compared to water/oil system, PEG environment involves low interfacial tension with a range of 10^{-2} mN/m to 1 mN/m shown in Table 4.2. Hence, the value of interfacial tension related with inertial and viscous force is necessary to stimulate the interfacial instability of aqueous phase jet [104][109][110]. As we considered above section, typical design microchannels do not utilize to make water-in-water (w/w) droplets from ATPS with low interfacial tension. Other studies used two different methods to stimulate breaking up of aqueous jets into monodisperse drops. Hydrodynamics perturbation is induced for droplet formation. External vibrators, such as piezoelectric actuator [87][115] or mechanical vibrator [90][116], are implemented to disperse jet flows of PEG. Due to the growth of Rayleigh-Plateau in stability by external force applying amplitude of perturbation, the corrugated jet can be controlled by frequency illustrated in figure 1.

Electro-hydrodynamic force is also utilized for droplet formation in microfluidic device. By applying DC electrical field, aqueous dropet (w/w) is split off from viscous jetting flow because two incompatible solutions have different electrophoretic mobility shown in figure 2 [117].

Table 3 List of the maximum interfacial tension values of different aqueous two-phase systems documented in literature. Interfacial tension between two immiscible aqueous two phases increases with the concentration of incompatible solutes of ATPS. The PEG/salt system typically has a relatively large interfacial tension (10^{-1} - 1 mN/m) and low viscosities (<20 mPa-s); while the protein/polysaccharide system typically has an ultra-low interfacial tension (≤10^{-2} mN/m) and a relatively high viscosity (> 50 mPa-s)

PEG/salt system [111]	PEG/Na_3CO_3	PEG/K_2HPO_4	PEG/Na_2SO_4
	1.99 mN/m	1.19 mN/m	0.80 mN/m
PEG/polysaccharide system	PEG/dextran [25]	PEG/maltodextran [112]	
	0.35 mN/m	0.12 mN/m	
Protein/polysaccharide system	gelation/dextran [113]	Sodium caseinate/ Sodium alginate [114]	
	0.03 mN/m	0.02 mN/n	

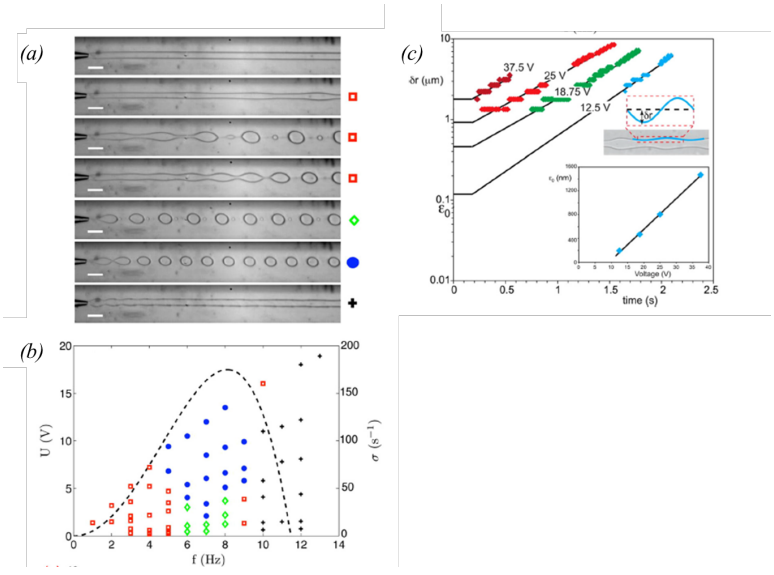


Figure 4.1 Forced breakup of a w/w jet induced by hydrodynamics perturbation. (a) and (b) Breakup bebaviors of a w/w jet triggered by forced oscillations at different perturbation frequency, *f*. Within an optimal range of frequency, monodisperse w/w droplets are generated, without stellite droplets, as shown by the blue dots [87][90].

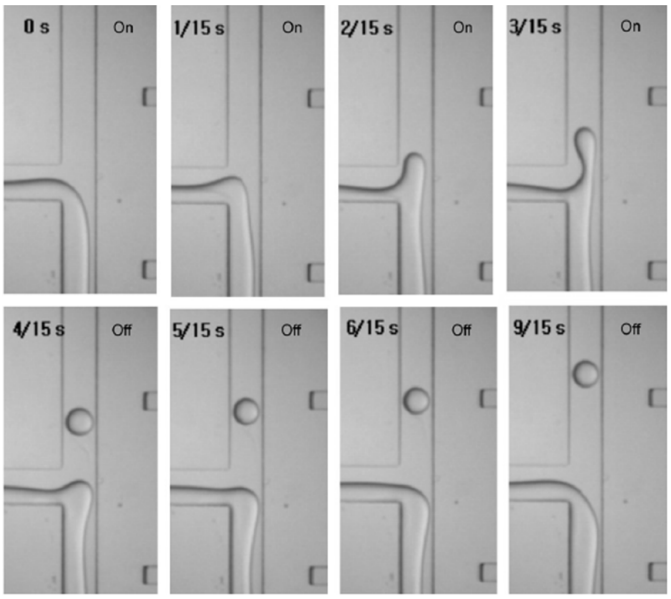


Figure 4.2 Formation of w/w emulsion induced by electro-hydrodynamic chopping [117].

In this section, as we previously accounted for the possibility of monodisperse dextran particles generated from double-flow-focusing, same approach can be employed for uniform PEG droplet emulsion. Poly(ethylene glycol) (PEG Mw 8000, Sigma-Aldrich Co., St. Louis, MO, USA) and dextran (Mw 500 000, Sigma-Aldrich Co., St. Louis, MO, USA) were used as immiscible aqueous phases in microfluidic device. PEG and dextran were dissolved into deionized water and Phosphate buffered saline (PBS) (10x, Sigma-Aldrich Co., St. Louis, MO, USA) to be 20 and 40 w/w % PEG, and 10 and 30 w/w % Dextran, respectively. Since the forced shear stress developed between two junction areas with laminar flow in double-flow-focusing design makes it possible to generate monodispersed dextran droplet, PEG droplet process can be expected in same manner. In double-flow-focusing channel, PEG phase (disperse) and dextran phase (continuous) form laminar flow. Depending on the combination of the ratio of PEG rate and two dextran rates, PEG jet is manipulated.

Thus, for phase partitioning, it should be considered for phase partitioning in microchannel to handle two immiscible. When PEG flow, dispersed phase, meets dextran flow, continuous phase, the phases are divided with the presence of focusing flow. When disperse phase moves on downstream, pressure-driven pumps change a unit volume of phases, and then the phase partitioning is not appealed at the end of channel. With the same flow rate of second dextran, the pressure ratio of PEG and first dextran present the status of two-phases in double-flow-focusing. Although droplets are developed at the ratio of pressure between 2.35 and 4.11, the interface of droplets in downstream has destroyed due to low interfacial tension, viscous force, and phase change across binodal curve. At small Capillary number, the droplet formation is highly depended on interfacial tension and viscosity [118][119]. However, viscoelasticity due to low interfacial tension and phase concentration depending on binodal curve should be deal with droplet-based microfluidics using aqueous two-phase system. When droplets are generated within upstream (between two junctions), the phase in downstream begins squashing the interface of droplets. In unit volume of microchannel, PEG phase concentration is not enough to form phase partitioning shown in figure 3 and then, dextran-rich phase is remained in downstream. Therefore, doubt-flow-focusing approach has alternative methods for water-in-water droplet monodispersity but, it cannot avoid the interface collapse in channel.

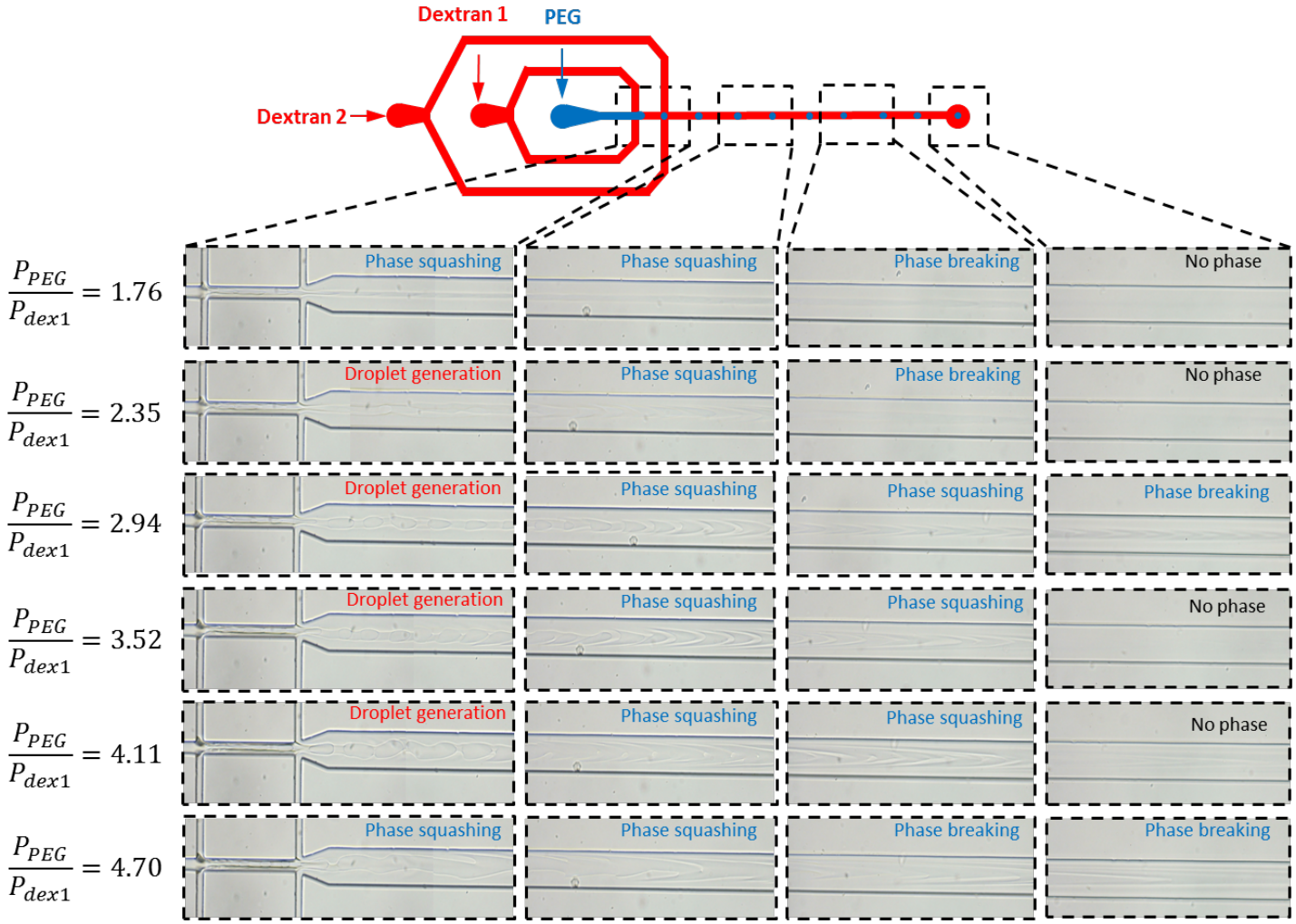


Figure 4.3 PEG droplet manipulation through double-flow-focusing. The pressure of dextran 2 phase is 100 mbar and the ratio of pressure of dextran 1 and PEG are conducted from 1.76 to 4.70. The regime of droplet generation is between 2.35 and 4.11 and other condition makes two phases single phase across bionodal curve.

4.3 Surfactant Effect on ATPS Droplet Generation

Surfactants are an important portion of the droplet-based microfluidics. The purpose of the use of surfactant involves the stabilization of droplet interface when they apply for biocompatibility of the system and in the process of molecular transfer between droplets [120]. When solution contains surfactant, the role of surfactant is to reduce the interfacial tension between the dispersed and continuous phases, thus facilitating surface deformation. However, surfactant affects the phase behavior and they a little increase interfacial tension in ATPS [121][122]. In viscous fluid system, the drag force on the droplet is intermediate between solid bodies [123]. Surfactants are generally utilized to stabilize emulsion droplets to avoid coalescence in microfluidics. Hence, PEG droplets in double-flow-focusing are expected by using surfactant impact on droplet emulsions. We prepared high concentration of polymer solutions enable to conjecture high interfacial tension. Poly(ethylene glycol) (PEG Mw 8000, 40% w/w, Sigma-Aldrich Co., St. Louis, MO, USA) and dextran (Mw 500 000, 30% w/w, Sigma-Aldrich Co., St. Louis, MO, USA) were used as immiscible aqueous phases in microfluidic device. PEG and dextran were dissolved into deionized water and Phosphate buffered saline (PBS) (10x, Sigma-Aldrich Co., St. Louis, MO, USA) Surfactant, TWEEN 80 (Sigma-Aldrich Co., St. Louis, MO, USA) (1 wt %) within dextran 10 % solutions, prevent the coalescence of the droplet. With the absence and the presence of surfactant, PEG droplet generation is appraised within double-flow-focusing. In high concentration of aqueous solutions leading relatively large interfacial tension in ATPS, the disperse phase cannot appeal to continuous phase apart from the range of pressure ratio between 2.68 and 3.5. Besides, PEG phase in the downstream did not sustaining its phase partitioning. The certain range

of flow rate ratio made it possible to form two-phase system and polydispersed droplets. With surfactant, dispersed phase is jetting flow within continuous phase as two-phase system at any range of flow rate. Apart from those range of flow rate, transition from jetting to dripping region for monodispersity of PEG droplets was not occurred. However, currently, surfactant in aqueous two-phase system has another consequence. As previous work resulting in interfacial tension increase in ATPS, the disperse phase kept jetting flow as two-phase system shown in figure 4. Therefore, while surfactant has played a vital role in droplet-based microfluidic using water/oil solutions, it is more required for aqueous two-phase system microfluidics to complement the use of surfactant and its characterization. It is because that surfactant has an effect on stabilization of droplet interface [120] and the control of interfacial properties of different phases brings the opportunity of multiple emulsions.

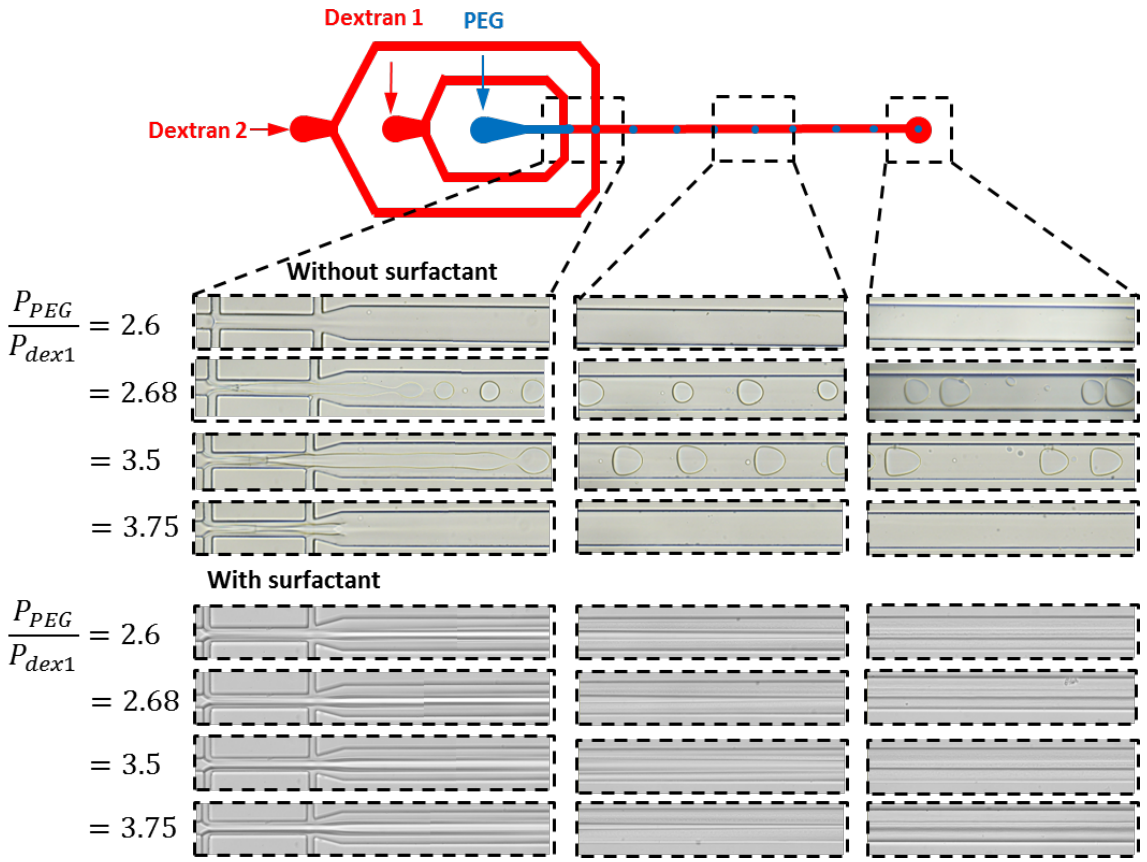


Figure 4.4 Droplet production in double-flow-focusing microfluidic chip. The polydispersity of the droplets are emerged within two-phase region outside binodal curve. With surfactant, the dispersed jetting flow continues to downstream without corrugation.

Chapter 5

Conclusion

Droplet-based microfluidics plays a vital role in miniaturization of the traditional chemical process and biological synthesis. Water and oil phases are mostly used for droplet-based microfluidics. However, even biocompatible oil is not able to supply biocompatible environment as water to biomolecules such as cell, bacteria, or protein. Therefore, aqueous two-phase system (ATPS), which supplies more eco-friendly environment for biomolecules than water and oil system, would be appropriate system. When ATPS is applied to the microfluidics, it involves a number of advantages. Low interfacial tension, for example, is appropriate for mass transfer. ATPS microfluidics allows easy purification and separation in microchannel.

In this thesis I designed the double-flow-focusing microfluidics to produce uniform water-in-water droplets. I enforced additional junction with popular method, called “flow-focusing” type, that two continuous phases changed the pressure distribution between two junction areas when disperse phase injected at certain combination of flow

rates. Depending on the combination of flow rates of disperse phase and two continuous phases, consistent or random size droplets were produced. Increasing disperse phase flow rate, transition from dripping to jetting flow occurred and deviation of droplet size was larger. Droplet size can be adjusted with first continuous phase and monodispersed region relied on second continuous phase. Droplets between 42 - 55 μm of dextran and 27 - 35 μm of PEG could be generated.

It observed that both dextran and PEG uniform droplets can be generated in double-flow-focusing which stimulate instabilities of interface in two phases. It demonstrated that the interface of an ATPS, as viscoelastic fluids, was much complex than water and organic solvent system and interfacial properties of ATPS was important for droplet generation.

In this study, monodispersed dextran droplets can be firstly formed through double-flow-focusing without external equipment. Monodispersed dextran and PEG droplets can be generated upon suitable flow rates combination, however, sizes of droplets are barely controlled. Besides, the various approaches for ATPS droplet generation in this thesis are expected to provide a platform for many research fields, such as drug delivery, cell analysis, and protein extraction.

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