An investigation on microfluidic droplet formation, reagent addition and mixing

Sivasamy Jayaprakash

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AN INVESTIGATION ON MICROFLUIDIC DROPLET FORMATION, REAGENT ADDITION AND MIXING

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Abstract

Significant efforts have been targeted towards developing a droplet-based microfluidic system and understanding the droplet formation process and the physics behind it and yet the underlying physics of the droplet formation in terms of evolution of pressures in both the continuous phase and dispersed phase has received less attention over the years and is not understood clearly. A numerical investigation on the mechanism of droplet formation in a microfluidic T-junction has been conducted and validated against the experimental flow visualisation. From the computational results it is shown that the pressure profile of the dispersed phase and the continuous phase in the squeezing regime changes as the droplet break-up process proceeds. New insights on the droplet break-up process such as the minimum pressure difference between the continuous phase and the dispersed phase happens at the last moment of the the droplet break-up and not during the second and third stage of the droplet formation mechanism in the squeezing regime have been identified.

As a next step in the analysis, reagent addition to microfluidic droplets is a fundamental operation in high-throughput screenings. Adding precise amount of reagents to droplets poses difficulties and many of the available designs use complicated structures and fabrication methods. A design that reliably adds reagents into droplets by exploiting the physics of fluid flow at an expanded section right after the T-junction which enhances merging of a stream with a droplet, eliminates the drawbacks such as extra droplet formation and long mixing time is demonstrated. Experimental results show that the expanded section minimizes the tendency to form extra droplets; the reactants are in axial arrangement inside the droplets which leads to faster mixing; reliable addition of reagent to the droplets happens for the combination of flow rates in a broad range of both substrate and reagent streams. To understand the physics behind the reliable operation of reagent addition, a numerical investigation using VOF model has been conducted. The flow field and the pressure measurements show that

when an expanded section is used, the dynamics of droplet formation mechanism undergoes a significant change and is dominated by the combination of both shear stress and pressure buildup-based droplet breakup from a pure pressured buildup-based droplet breakup in the squeezing regime; and the expanded section plays the role in creating low Laplace pressure jump across the interface of the droplet forming from the T-junction which reduces the probability of forming extra droplet in the merging process.

After the addition of reagents to the droplets, the contents need to be mixed as fast as possible to reduce the process time to have a high throughput. Microchannel designs for adding reagents to droplets and promoting droplet micromixing have been reported but the strategy behind the designs are basically intuitive and there is a lack of design methods based on first principles. A simple method for evaluating chaotic advection in slug micromixing is proposed. In this method, a slug moving in a slit microchannel ($(w \gg h)$) and flow field in a plane far from the boundary walls is modelled as two-dimensional low-Reynolds-number flow (Stokes flow). The two-dimensional analytical solution is used to track massless passive tracer particles by applying boundary conditions which mimic the motion of the slugs in microchannel geometries, in Lagrangian frame of reference. Poincaré sections and dye advection patterns are used to analyse chaotic advection of passive tracer particles using statistical concepts such as ‘Variance’, ‘Shannon entropy’ and ‘Complete spatial randomness’. An optimization exercise has been carried out to find the optimal operating variables for faster droplet mixing in the meandering microchannel. This method has been applied to find a new channel design and the movement of the droplet in the microchannel geometry resembles an oscillating droplet and the mixing operation has been found to enhance chaotic advection in microfluidic droplets.

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Nomenclature

$\mu - TAS$	Micro Total Analysis Systems
Re	Reynolds number (dimensionless)
ν	kinematic viscosity (m^2/s)
D_{mol}	diffusion coefficient (m^2/s)
Pe	Peclet number (dimensionless)
Ca	Capillary number (dimensionless)
p_d	pressure measured in the dispersed phase (N/m^2)
p_c	pressure measured in the continuous phase (N/m^2)
$p_d - p_c$	pressure difference between the dispersed
and the continuous phase (N/m^2)	
Δp_L	Laplace pressure (N/m^2)
h_c	critical distance (m)
V_{rel}	relative velocity (m/s)
$V_{particle}$	particle velocity (m/s)
I_{var}	variance index (dimensionless)
I_S	entropy index (dimensionless)
σ^2	variance (dimensionless)
$I_{var,CSR}$	the CSR limit for variance index (dimensionless)
$I_{S,CSR}$	the CSR limit for entropy index (dimensionless)
ρ	density (kg/m^3)
μ	dynamic viscosity (Pa.s)
p	pressure (N/m^2)

CFL	Courant number (dimensionless)
t	time (s)
Δt	time step value (s)
Δx	mesh size (m)
Q_{fluo}	flow rate of DI water with fluorescence ($\mu\text{l/hr}$)
Q_{DI}	flow rate of DI water ($\mu\text{l/hr}$)
R_w	interface curvature in the direction of the width of the channel (m)
R_h	interface curvature in the direction of the height of the channel (m)
V_{cell}	volume of a computational cell
F	liquid volume fraction in a cell (dimensionless)
$\vec{F}_s$	volumetric force due to surface tension (N/m^3)
U_{top}	velocity of the top wall (m/s)
β	aspect ratio (dimensionless)
U_{bottom}	velocity of the bottom wall (m/s)
Ω	Angular velocity (rad/s)
P_{cap}	Capillary pressure (Pa)
σ	Surface tension coefficient (N/m)
α_q	q th fluid's volume fraction in the cell (dimensionless)
κ	surface curvature (m)
n	surface normal (dimensionless)
θ_w	contact angle (radians)
PDMS	polydimethyl siloxane
PTFE	Polytetrafluoroethylene
VOF	Volume of Fluid
CSF	Continuum Surface Force
$CaCl_2$	Calcium Chloride

A	Hamaker Constant (dimensionless)
PAA	polyacrylic acid
h_c	critical distance (m)
μ -PIV	Micro-particle Image Velocimetry
RTD	Residence Time Distribution
PSD	Particle Size Distribution
$V_{particle}$	Velocity of Particle (m/s)
CSR	Complete Spatial Randomness
PMF	Probability Mass Function
N	number of particles
M	number of bins

1. Introduction

1.1. Microfluidics — Background

Microfluidics is the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens and hundreds of micrometres [30, 31]. The field of microfluidics is interdisciplinary which takes motivation from fluid mechanics, molecular analysis, biodefence, molecular biology and microelectronics. Microfluidics exploits the ability to create structures and patterns in microscale dimensions that comes from microelectronics; and subsequently evolves the techniques for manipulating and controlling fluids at microscale dimensions on the principles of fluid mechanics, molecular analysis and molecular biology. This remarkable technological merging of diverse fields has triggered a wide range of scientific investigations as well as the development of devices in the fields of biotechnology, analytical chemistry and combinatorial chemistry. Handling tiny volumes of liquids from nanolitres to few microlitres and transporting and manipulating them in microscale dimensions are filled with challenges that are very different from what is encountered in the macroscale.

Considerable research has been devoted to the development of components such as micropumps, microvalves, mixing chambers and detection units. An integration of these miniaturized components leads to the so-called “lab-on-a-chip” and is revolutionizing the chemical, biochemical and healthcare industry. In addition to that the microfluidic based systems have dramatically changed the way in which traditional medical laboratories work: they are smaller, cheaper, faster, portable, fully automated and make accurate measurements than it were possible a decade ago. Application of these microfluidic systems ranges from monitoring patients in remote areas, monitoring of air and water quality and other environmental factors. Microfluidic applications in these areas have been made possible by the physical effects of handling fluids in microscales — the fundamental fluid flow physics is vastly

Table 1.1. *Scaling effect of transport properties [29]*

	nm	μm	mm	m
Length (L)	10^{-9}	10^{-6}	10^{-3}	1
Surface area (L^2)	10^{-18}	10^{-12}	10^{-6}	1
Volume (L^3)	10^{-27}	10^{-18}	10^{-9}	1
Specific surface area (L^{-1})	10^9	10^6	10^3	1
Rate ($\propto L$)	10^{-9}	10^{-6}	10^{-3}	1
Inertial force ($\propto L^4$)	10^{-36}	10^{-24}	10^{-12}	1
Viscous force ($\propto L^2$)	10^{-18}	10^{-12}	10^{-6}	1
Interfacial tension ($\propto L$)	10^{-9}	10^{-6}	10^{-3}	1
Viscous force/inertial force ($\propto L^{-2}$)	10^{18}	10^{12}	10^6	1
Interfacial tension/inertial force ($\propto L^{-3}$)	10^{27}	10^{18}	10^9	1

different when the length scales become smaller.

Table 1.1 lists how the relative magnitudes of transport properties and forces change from macroscale to microscale. It can be seen that fundamental quantities like surface area, volume and transport properties such as inertial force, surface forces etc., change sharply as length scales become smaller ([32, 33]). The designs for microscale fluidic systems are strongly influenced by the effects of high dissipation rates, decreased volume-to-surface area ratio (e.g., surface tension phenomena), and electrohydrodynamic phenomena. Also, miniaturized fluidic devices provide the capabilities of performing procedures that are extremely difficult or impossible to execute in macro scale ([34, 35]).

1.1.1. Lab-on-a-Chip Devices

In Lab-on-a-Chip (LOC) or Bio-Microelectromechanical Systems (Bio-MEMS) a complete chemical analysis is done in different stages at the component level in an integrated and automated fashion. These stages include sampling, sample pre-treatment, chemical reactions, analytical separation, production isolation and data analysis. Among these individual components, the units for adding and mixing samples with reagents are critical for the subsequent chemical reactions and detections. The reagent addition unit has to mix appropriate amount of reagent to the sample and the mixing unit has to provide sufficient mixed solution in a confined length of a micro-device, and before the point where detection is taking place. Mixing issues are complicated, and sometimes counterintuitive, because viscous effects dominate at small scales and viscosity-dominated flows are deterministic [36]. It can be noticed in table 1.1 that in microspaces, viscous force becomes dominant and the resulting flow in the

microchannel remains laminar, Fig. 1.2.

Figure 1.1. *Picture of a plastic LOC device for point-of-care clinical diagnostics [1]*

1.1.2. Fluid flow in microfluidic devices

In microchannels viscous force predominates and the flow is invariably laminar with Reynolds number ($Re = Vd/\nu$ (V is the average flow velocity, d is the characteristic cross-sectional channel dimension and ν is the kinematic viscosity of the fluid) well below the threshold value for turbulence, which is usually > 2100 for macroscale. Furthermore, values of the Peclet number are relatively high in microchannels for biological materials whose diffusion coefficients are in the order of $D_{mol} = 10^{-5} \text{ cm}^2/\text{s}$ ($Pe = Vd/D_{mol} \geq 100$, where D_{mol} is the molecular diffusivity). Therefore, mixing in microfluidic channels which occurs due to diffusion happens at a much slower rate than the timescales associated with fluid motion. These factors combine to produce characteristic mixing lengths ($\Delta y_m V * (d^2/D_{mol}) = Pe * d$) on the order of several centimetres, resulting in the need to employ clumsily long channels in order to achieve complete mixing. In such viscous flows, even though one can put mechanical agitation into a microfluidic device, it is not likely to efficiently improve mixing performance [37]. As a sequence of these limitations, considerable effort has been directed toward the development of strategies to achieve rapid laminar flow mixing in microfluidic systems [38].

Figure 1.2. *Laminar flow in a microchannel. Aqueous streams labelled with blue and yellow food dyes are entering the channel and the streams flow parallel to each other in the absence of any turbulence [2]*

1.2. Droplet Microfluidics

Droplet-based microfluidic systems focus on creating discrete volumes with the use of immiscible phases, unlike continuous microfluidic systems. But possesses the important characteristics of microfluidic systems such as low-Reynolds number flow regime, which dictates that all fluid flow is essentially laminar, precise control of reagents and reactions and high-throughput fabrication of the devices [39]. Droplet-based microfluidics is a new paradigm shift in microfluidics research for the fact that many chemical and biological agents are contained in microdroplets [40].

Droplet-based microfluidic systems are being studied widely because they are useful as sample transporters, mixing enhancers, dispersion eliminators and simply good discrete microreactors [19, 41, 25, 42, 43, 39]. The importance and versatility of droplet microfluidic systems lie essentially in the ability to transport and precisely dial-in fluid volumes of each individual droplet. This, coupled with the precise generation and repeatability of droplet operations, has made the droplet-based microfluidic system a high throughput platform for biomedical research and applications. In addition to being used as microreactors ranging from the nano- to femtoliter range, droplet-based systems have also been used to directly synthesize particles and encapsulate many biological entities for biomedicine and biotechnology applications. Liquid/liquid two phase systems offer the possibility of complex sample handling

in chemical, biomolecular and microbiological operations using the special flow behavior of embedded liquid segments.

Therefore, droplet microfluidics has received significant attention over the last decade in the areas of point-of-care testing, DNA separation, drug delivery, cosmetics, food and many other inexpensive diagnostic tools [4, 44, 9, 45, 46, 47, 8, 48]. In droplet microfluidics, basically there are three steps involved in an application: 1) droplets are generated in an immiscible carrier fluid using methods that are conducive in the microscale dimensions [3]; 2) after generating the droplets reagents are added appropriately for a particular analysis; 3) down the line, after mixing, the ingredients of the droplets are analysed with suitable techniques. Each one the above operations is crucial in any diagnostic technique and carrying out them in microscale dimensions and in real-time integrated applications raises difficulties in realizing reliable complex droplet based lab-on-chips. Therefore, in this thesis we focus on developing methods, design and knowledge on droplet generation, reagent addition and droplet micromixing in microfluidic channels.

1.3. Outline of the thesis

Chapter 2: Literature Review

In this chapter, a brief overview of droplet based microfluidics and its usefulness as a candidate for “lab-on-a-chip” operations is presented. A comprehensive literature review on droplet generation methods and the mechanism of droplet formation and methods for reagent addition to droplets are presented in the first section of the chapter. And in the second section of the chapter a review on droplet micromixing is presented. Results reported by other researchers on the above are critically analysed.

Chapter 3: Microfabrication Procedures and Experimental Setup

In this chapter, details of the microfabrication of microfluidic channels through soft-lithography and the experimental setup used for the experiments are presented.

Chapter 4: An investigation on the mechanism of droplet formation in a microfluidic T-junction

In this chapter, findings of an experimental and numerical investigation on the mechanism of droplet break-up in a microfluidic T-junction are presented. Droplet formation process and the evolution of pressure in the dispersed phase and the continuous phase in

the squeezing regime, transition regime and dripping regime are investigated. New insights on the pressure difference between the dispersed phase and the continuous phase during the droplet break-up process are presented.

Chapter 5: Merging Reagents into Droplets - Experimental Results

In this chapter, results of an experimental investigation of a new microchannel design for reliably adding reagents into preformed droplets at a T-junction are reported. The physics behind the reliable operation of reagent addition into the sample droplets for a wide range of reagent flow rates is explained.

Chapter 6: Merging Reagents into Droplets - Computational Results

In this chapter, results of a numerical investigation on the reliable operation of reagent addition into droplets at a T-junction and the physics behind it are presented. The numerical results are compared against the experimental flow visualization to validate the findings.

Chapter 7: A simple method for evaluating and predicting chaotic advection in microfluidic slugs

In this chapter, a new method to evaluate and predict whether a particular microchannel design can develop chaotic advection or not is proposed and the results of a numerical investigation are reported. An optimization exercise carried out to find the optimal operational parameters such as aspect ratio and velocity of the droplet in the microchannels for enhanced mixing is presented. Using the method proposed here, a new microchannel design has been identified for enhancing chaotic advection, based on the boundary conditions which mimics an oscillating droplet.

Chapter 8: Conclusions and Future Research Directions

In this chapter, conclusions of the current investigation are presented and suggestions for future research directions are given.

2. Literature Survey

2.1. Introduction

Microfluidic systems developed with multiphase flows are used for miniaturizing chemical and biological laboratory techniques [39]. Fundamental and applied research in chemistry and biology benefits from opportunities provided by droplet-based microfluidic systems. Compartmentalization in droplets provides rapid mixing of reagents, control of the timing of reactions on timescales from milliseconds to months, control of interfacial properties, and the ability to synthesize and transport solid reagents and products. Droplets help to enhance and accelerate chemical and biochemical screening, protein crystallization, enzymatic kinetics, and assays [42]. In this chapter, the use of droplets in the field of microfluidics is comprehensively reviewed and presented in the sequence from droplet formation, reagent addition to droplets and droplet mixing in microchannels.

2.2. Droplet Formation in Two-Phase Flows

Aqueous droplets in microchannel are usually generated in a immiscible carrier fluid using T-junction or flow focusing channel [46, 49]. Miscible fluids would flow alongside each other and diffuse freely from one stream to the other. However, for immiscible fluids, the presence of the surface tension (σ) would affect the dynamics of the free surface. The stress exerted by the surface tension is so significant that causes free surface deformations and/or bulk liquid motion. Microfluidic methods for forming droplets can be either passive or active. Most methods are passive, relying on the flow field to deform the interface to promote the natural growth of interfacial instabilities. Passive methods can be grouped into three categories, characterized by the nature of the flow field near pinchoff: (1) breakup in co-flowing streams, (2) break up in cross flowing streams and (3) breakup in elongational or stretching dominated

flows [3]. Schematic illustrations of these three geometries are shown in Fig. 2.1.

Figure 2.1. *Illustrations of the three main microfluidic geometries used for droplet formation. (a) Co-flowing streams, (b) crossflowing streams in a T-shaped junction, and (c) elongational flow in a flow focusing geometry [3]*

Thorsen et al.[4] first observed the formation of aqueous droplets in oil in microchannels as seen in Fig. 2.2. The authors suggested that the dynamics of droplet formation is dominated by the balance of tangential shear stresses and interfacial tension (i.e the capillary number).

Tice et al. [5] characterized the experimental conditions required to form nano-liter sized droplets of viscous aqueous reagents in flows of immiscible carrier fluid within microchannels. They reported that plugs formed reliably in a flow of water-immiscible carrier fluid for Capillary number less than 0.01 as seen in Fig. 2.3.

Tan et al. [6] implemented another flow-focusing geometry design to perform droplet formation in the two-phase liquids. When two immiscible liquids are forced through an orifice, a liquid thread will braid inside the microchannel, the point of droplet breakup varied inside the microchannel and the breakup over the same length of the liquid thread did not always give rise to the same number of droplets. An expanding nozzle was designed to control the breakup location to one single point located at the orifice. This is because the narrowest point incurs the highest shear force. So the expanding nozzle (Fig. 2.4) was used to create

Figure 2.2. *Microfabricated channel dimensions at the point of crossflow and photo-micrograph of water introduced into the continuous oil-surfactant phase [4]*

the narrowest point to focus and dissipate the force of the flow. The expanding nozzle design allows the fluid to accelerate and reach maximum velocity before entering the nozzle and follow by decreasing at the exits of the orifice. As the result, a high velocity gradient around the orifice of the nozzle is created and this allows the droplet to break continuously at the location of maximum shear stress and pressure point.

Nisisako et al.[7] used a controllable three-stream laminar flow system to hydrodynamically focus a viscous stream, flowing between two immiscible streams, to cause instability and eventually detach from the original stream. This study aimed to overcome the dependence of droplet size on channel size and to generate highly monodisperse droplets with precise control of size and production rate. The flow focusing geometry design was different from previous study as the inlet channels were tapered down near the junction of the three inlet channels (Fig. 2.5). The two outer liquid streams met the mid liquid stream at the junction at a 60° where they entered into the main channel. The dispersed phase liquid flowing out from the central channel into the side channels, if the flow of rate of the continuous phase was not kept at a higher value than that of the (dispersed phase) to a critical width to cause the Rayleigh

Figure 2.3. *Visualisation of the three regimes of behaviour observed in two-phase flows of viscous liquids. (a) Left: A scheme of the microfluidic network. Right: a micrograph of formation of plugs at low values of $Ca = 4.7 \times 10^{-3}$. (b) A microphotograph of flow that is in transition to laminar flow. (c) A microphotograph of flow where the length of the laminar segment is 200μ [5]*

instability and detachment of the central stream.

In their study, the width of the central stream gradually decreased as the F_s/F_c (flow rate of side flow to central flow) increased. However, at a certain F_s/F_c the width decreased with increasing F_s . Whenever the width was thinned below $3.5 \mu\text{m}$ the central stream breaks up. The higher the flow rate of the central stream, the flow rate ratios required for achieving the critical break up width will be lowered. This showed that the flow rate of the central stream affected both the narrowing and the detachment of the central stream. If the flow rate ratio went beyond the critical flow rate at a certain central stream flow rate, the droplet formed would be in irregularly shaped due to unsteady breakup.

Nisisako et al.[7] proposed a microfluidic device having both hydrophobic and hydrophilic components that is exploited for production of multiphase emulsions (double emulsion). A double emulsion can be defined as a multiphase dispersion in which droplets enclosing finer droplets are suspended in a continuous liquid phase. Generally, there are 2 type of double emulsions which are water-in-oil-water ($W/O/W$) and oil-in-water-oil ($O/W/O$) have the attracted considerable attention because of there potential application in food, cosmetics and pharmaceuticals. For producing $W/O/W$ dispersions, aqueous droplets ruptured at the upstream hydrophobic junction are enclosed within organic droplets formed at the downstream hydrophilic junction. It was because the hydrophobic microchannels are suitable for dispers-

Figure 2.4. (a) Close up images of the narrowing down of the central stream near the channel junction (b) Effect of the flow rate ratios F_s/F_c on the narrowing of the central stream at the exit of the channel junction at different F_c . The width was measured from microscopic images after the flows reached a steady state [6]

Figure 2.5. Basic concept for preparing double emulsions (W/O/W) in T shaped microchannels ([7])

Figure 2.6. *Schematic of the coaxial microcapillary fluidic device. The geometry requires the outer fluid to be immiscible with the middle fluid to be in turn immiscible with the inner fluid ([8])*

ing water drops in an organic phase (oil), as for the hydrophilic microchannels are suitable for dispersing organic drops in water phase. It is able to suspend more than 1 droplets inside a single organic or water droplets as presented.

Utada et al.[8] generated double emulsions from hydrodynamic focusing coaxial jets in a single step, allowing precision control of the outer and inner drop size as well as the number of droplets encapsulated in each larger drop. The innermost fluid is pumped through a tapered cylindrical capillary tube and the middle fluid is pumped through the outer coaxial region (Fig. 2.6), which form a coaxial flow at the exit of the tapered tube. The outermost fluid is pumped through the outer coaxial region from the opposite direction and all fluids are forced through the exit orifice formed by the remaining inner tube. The flow passed through the exit orifice and subsequently ruptures to form drops.

De Menech et al.[50] identified, through a numerical investigation of the dynamics of break-up of immiscible fluids at a microfluidic T-junction, three distinct regimes of formation of droplets: squeezing, dripping and jetting. They used the phase-field model to numerically compute the pressure, droplet volume and droplet radius and based on the observation they identified the three regimes in the dynamics of droplet break-up.

Christopher and Anna[51] reported a systematic experimental study of droplet break-up at T-shaped microfluidic junctions for conditions near the transition from squeezing dominated to dripping, where the viscous stresses become important. They described the complicated process based on two dimensionless parameters: the capillary number and the flow rate ratio. Xu et al.[52] proposed correlations for droplet formation in T-junction ranging from

squeezing to dripping and developed a modified capillary number for the continuous phase by using the local continuous phase flow rate at the droplet formation site.

Sang et al.[53] studied the effect of viscosity, for both Newtonian and power law fluids, on droplet formation in T-shaped microchannels by analytical and numerical methods. Liu et al.[54] performed a numerical study using phase-field model to describe fluid/fluid interfacial dynamics and a lattice Boltzmann model to address hydrodynamics to understand the mechanisms of droplet formation in a microfluidic T-junction.

In general, the fluid phase to be dispersed is driven into a microchannel via a pressure-driven flow in which either volume flow rate or applied pressure are controlled. A second immiscible liquid is driven into a separate microchannel via an independently controlled flow. The two streams meet at a junction, at which the dispersed phase liquid extends to form a finger or jet. The geometry of the junction and the volumetric flow rates of the two fluids determine the local flow field, which deforms the interface. Eventually, a droplet pinches off from the dispersed phase finger by a free surface instability. Steady flow of the two liquids yields periodic formation of equal-size droplets in a continuous stream. Generically, droplet breakup can be characterized by the competition between local fluid stresses acting to deform the liquid interface and capillary pressure acting to resist deformation. In addition, the emerging droplet obstructs the junction as it grows, leading to a dramatic increase in the upstream pressure, which also drives pinchoff. The wettability of the nearby channel walls is critically important to the process, determining which liquid phase is dispersed. Size of the microfluidic droplets can be varied by changing the flow rates of immiscible fluids ([55, 3]).

2.2.1. Mechanism of droplet breakup

Garstecki et al. [49] described the process of formation of droplets and bubbles in a microfluidic T-junction. They identified that at low capillary numbers break-up is not dominated by shear stress but by the pressure drop — the dominant contributor to the dynamics of break-up — across the emerging droplet and named the mechanism as squeezing. They argued that this mechanism is directly connected to the confined geometry in which the drop is formed and proposed a scaling law for the size of the droplets that is based only on the ratio of flow rates of the two immiscible liquids and independent of the value of the capillary number.

They argued that the dynamics of droplet break-up in a typical T-junction is dominated by the balance of pressures in the dispersed (p_d) and the continuous (p_c) phases at the

junction. They drew a heuristic picture of the break-up process as seen in Fig. 2.7 and postulated a mechanism based on the assumption that the pressure in the dispersed phase at the inlet remains constant throughout the break-up process (long-dashed line in Fig. 2.7(e)) and explained the process by the evolution of the continuous phase pressure (p_c). Though the assumption essentially reduced the complexity and enabled the process to be explained in simple terms, it didn't capture the real scenario.

Though De Menech et al.[50] studied the continuous phase pressure p_c upstream of the T-junction to understand the droplet break-up mechanism from squeezing to dripping and jetting, they didn't study the dispersed phase pressure to completely explain the physics of the droplet break-up in terms of changes in the pressures of the two phases.

2.3. Performing Reactions in Droplets

2.3.1. Introduction

Droplet volumes ranging from femtoliter to microliter volumes compartmentalize the reagents and offer conditions to perform multiple reactions. Problems like evaporation, complicated fluid handling, dispersion, and diffusion can be overcome by using multiphase flows of immiscible liquids to form droplets in microfluidic channels.

To perform reactions within microfluidic devices, the microfluidic tool that is used should meet certain criteria. They are: 1) It should be able to perform typical procedures that are conducted for reactions on the macroscale. These procedures include the controlled addition of reagents to a reaction mixture, the thorough mixing of reagents, control of the reaction time, the combining and splitting of reaction mixtures for multiple-step reactions, and analysis over the course of a reaction. 2) It should provide a characteristic advantage, for example, the ability to perform more reactions under more reaction conditions. It should also provide a method for organizing and indexing each reaction condition. Also, there must be an efficient method for assaying many different conditions and also for optimizing a particular condition. And these methods should be scalable, straightforward, and simple.

Reagents must be introduced into droplets which is necessary for high-throughput screenings, where one target sample is tested against a large number of different reaction conditions ([42]). Each reaction condition may be composed of different reagents or a different combination of a set of reagents. For measuring kinetics or optimization reaction

Figure 2.7. (a) An illustration of the shape of the tip of the immiscible thread at an intermediate stage of break-up. Insets (a) and (b) illustrate the axial and radial curvature, and the positions at the hydrostatic pressures P_d and P_c in the discontinuous phases respectively. (c) Evolution of the Laplace pressure jump across the interface (Δp_L), and (d) four stages of formation of droplet: the stream of the discontinuous fluid enters into the main channel (I), the stream blocks the main channel (II), the droplet elongates and grows downstream (III), the droplet separates from the inlet (IV). (e) Schematic illustration of the evolution of the hydrostatic pressure p_d in the dispersed phase at the end of the inlet, pressure p_c in the continuous phase in the junction, and the difference $p_d - \Delta p_L$ ([9])

Figure 2.8. *The concentrations of the crystallizing reagents (PEG, buffer, protein, and NaCl) are varied by varying the relative flow rates of the reagents ([10])*

conditions, only a few reagents need to be incorporated within the droplet, but the concentration of these reagents are varied. Adding a precise amount of reagents to a droplet poses difficulties and various schemes for reagent addition have been investigated by researchers. This section critically analyzes the methods reported for reagent addition or merging reagents to the droplets in microchannels.

2.3.2. Methods of Reagent addition

Methods of reagent addition to droplets that have reported in the literature are: 1) injecting the reagents while the droplet is forming at the T-junction or otherwise called as direct injection of reagents into droplets; 2) merging a reagent stream from a T-junction into preformed droplets or catridge technique; 3) coalescing the two separately formed droplet through various external application of driving forces.

2.3.3. Introducing reagents into the droplet while it is forming

Song et al.[20] reported a method for producing droplets or plugs containing reagents by injecting the reagent streams together at a T-junction as shown in Fig. 2.8. In this method a solution of reagent A (PEG), a solution of reagent B (protein), a buffer and a solution of reagent C (NaCl) were injected as steady streams into a microfluidic channel at initial point $d=0$ where the reaction between them begins ($t = 0$). The reaction mixture is transported by the fluid stream at a constant velocity U and every spatial point d corresponds to a time point t and the reaction time $t = d/U$.

To form droplets from three solutions of reagents without bringing the reagents into prior contact they flowed these solutions in a microchannel as two laminar streams, and used an inert center stream to separate them. These four streams were continuously injected into

Figure 2.9. *Preformed cartridges of plugs enable the combination of large number of reagents with a sample in sub-microliter volumes. a) Four different reagents stored as an array of plugs in a capillary. The plugs are separated by a fluorocarbon carrier fluid, as well as air bubbles (in (b)), to prevent cross-communication between the plugs. c) Merging of plugs from a preformed cartridge with a target sample stream through a T junction. The resulting array of plugs is transferred into a receiving capillary and the trials are collected ([11])*

a flow of water-immiscible oil in the main microchannel, where they spontaneously broke up into streams of plugs (~ 500 pL) separated and surrounded by oil. Though it offers a simple method to inject reagents into droplets, the precise control of reagent composition in each droplet through adjusting the flow rates is unpractical in the microfluidic device since the asymmetric shear force ([56]).

2.3.4. Catridge Method

Zheng et al.[11] demonstrated a method for screening a large number of reaction conditions against one target sample in which they used a preformed droplets in a catridge to store an array of plugs. Each plug contained a different reaction condition of different reagent. For demonstration purpose, they formed an array of 48 plugs and each plug contained 15 nL of a different reagent. The target sample was introduced into the preformed plugs by using a microchannel T-junction as shown in Fig. 2.9.

Figure 2.10. *Preformed cartridges of plugs enable the combination of large number of reagents with a sample in sub-microliter volumes. a) Four different reagents stored as an array of plugs in a capillary. The plugs are separated by a fluorocarbon carrier fluid, as well as air bubbles (in (b)), to prevent cross-communication between the plugs. c) Merging of plugs from a preformed cartridge with a target sample stream through a T junction. The resulting array of plugs is transferred into a receiving capillary and the trials are collected ([11])*

2.3.5. Direct Injection of Reagents into Droplets

For multiple-step reactions, a reaction mixture is allowed to react for a certain time and then another reagent is added to the mixture. Using the method of introducing reagent streams to the droplet while it is forming can be the first step, and the reagent for the second step can be injected into the plug through a side channel further along the network. Or, the first step of the channel can be also be started by injecting the reagent after the droplet was formed.

Merging a stream into a droplet

Song et al.[12] developed a method to merge a stream into the preformed droplets in a microchannel as shown in Fig. 2.11. They injected $CaCl_2$ from the side hydrophobic channel into the blood plug flowing in the main channel and found that probability of successful merging is 92-99%. They replaced the side hydrophobic channel with hydrophilic to make side stream stick to the channel until the aqueous plug arrives at the junction for injection.

They observed consistent merging (100 %) when the side capillary was inserted into the main channel edge and the blood plugs were larger than the $CaCl_2$ droplets formed in the junction (Fig. 2.12). They controlled the amount exactly by controlling the flow rates. However, the fabrication effort required for this method compared to the above is significant as this method requires multistep fabrication unlike the single step softlithography for the side hydrophobic channel method. It also suffers from issues such as the need for synchronization of the flow of the substrate and cross-contamination of the plugs, and formation of extra

Figure 2.11. *Merging within a microfluidic device using a hydrophobic side channel. (a) When the side channel was hydrophobic (silanized PDMS), contamination occurred (for 5 out of 5 experiments) when the side channel was large (width of $200\ \mu\text{m}$ and height of $250\ \mu\text{m}$). (b) However, merging did not occur (for 4 out of 4 experiments) when the side channel was too small (width and height of $20\ \mu\text{m}$). Another approach for merging was to form droplets of CaCl_2 at the same frequency as the passing plug ([12])*

Figure 2.12. *Consistent merging with a hydrophilic glass capillary inserted into the side channel ([12])*

Figure 2.13. *Schematic of the A) A simple T-junction device B) multijunction injector with hydrophilic side channels. ([13])*

droplets of substrate when the plug arrival is not synchronized with the droplet formation from the side channel. It also operated in a very narrow range of flow ratios of the substrate and the reagents flow rates.

Li et al.[13] introduced a multijunction injectors with hydrophilic side channels to improve the injection of a substrate into an array of preformed plugs carried by an immiscible fluid in a microchannel as shown in Fig. 2.13. This method improved the injection of substrate to the preformed plugs without the synchronization of the flow of substrate and the array of preformed plugs of reagent, which reduced cross-contamination of the plugs, and eliminated the formation of small droplets of substrate, and allowed a greater range of injection ratios compared to that of a single T-junction.

Dosing of liquid reagents into droplets using a single T-junction in a microchannel was demonstrated by Henkel et al.[14]. They used single nozzle T-injectors for segment generation and continuous dosing of liquid to a continuous stream of segments as shown in Fig. 2.14. The T-injectors system was used for titration experiments of formic acid with disodium hydrogen phosphate solution. 10mM Formic acid in water, stained with 0.3mM bromophenol blue as indicator dye was used for segment generation at a flow rate of 1.5 ml for tetradecane and 0.3 ml h⁻¹ for formic acid. However, those demonstrations were limited to a few flow rates of the reagents.

Shestopalov et al.[57] reported a method to adding reagents to droplets in which they injected reagents directly into the droplets. Method of injecting the reagent with the sample to form droplets at the T-junction has difficulties in precisely controlling the amount of

Figure 2.14. *Dosing of sodium hydrogen phosphate to formic acid compartments, stained with bromophenol blue. (A) Immediately before a acid compartment reaches the nozzle with the elongated interface of the phosphate solution, (B) fusion of the interfaces, (C) dosing of buffer and (D) background reduced, enhanced image of the compartment immediately before tear off, the dark color in the frontal part indicates indicator switch ([14])*

reagents due asymmetric shear stress at the inlet boundary ([20, 43]). Mixing of reagents can happen in this method, before droplet formation, which interferes in the case of following instantaneous reactions and this method is not suitable for adding different reagents and carry out subsequent reactions. Method of coalescing the droplets by surface energy pattern ([16]) or geometry mediation ([17]) has been used to force the immiscible fluid in between the individual droplets and bring into contact to coalesce them.

2.3.6. Application of external forces

Droplet Coalescence using electrical force

Sarrazin et al.[15] studied the coalescence of microdroplets generated in a microchannel by polarizing them using electrodes of opposite charges as shown in Fig. 2.15. They used a dye and water, an acid-base instantaneous chemical reaction as an colored indicator of mixing and compared different angles of bended channels and different ways of coalescence and have shown that the homogenization of the droplets can be reached in less than 10 ms after coalescence by adding 45° angle bends along the channel.

They concluded that the presence of dead zones were observed at the back and front of the droplets and suggested to characterize the detailed hydrodynamics of droplet transport in microchannels through simulations and microparticle image velocimetry experiments and check the presence of different structures inside the drops. The main disadvantage of using electrostatic forces to coalesce the droplets, however, is not suitable for use with biological materials.

Figure 2.15. *Generation of multi-component droplets controlled by an electric field: opposite charged droplets are generated and transported in a continuous phase; they coalesce in the T-junction so that the resulting droplet can mix and react (side-by-side coalescence configuration) ([15])*

Figure 2.16. *Dye and water droplets flowing in a straight channel after side-by-side coalescence ([15])*

Figure 2.17. *Dye and water droplets flowing in a straight channel after side-by-side coalescence ([16])*

Fidalgo et al.[16] demonstrated a method for droplet fusion based on a surface energy pattern on the walls of a microfluidic device, that does not require active elements nor accurate synchronization of the droplets as shown in Fig. 2.16. They patterned hydrophilic polyacrylic acid (PAA) and grafted via UV photopolymerization on planar benzophenone-containing poly(dimethyl siloxane) (PDMS) substrates to trap the aqueous microdroplets. They described the surface induced droplet fusion in two subsequent processes: droplet trapping and droplet detachment and said coalescence will take place when the interfaces are closer than a critical distance h_c (in the order of nanometres) for a time longer than: $t = 96\pi^2\nu\eta h_c^5 A^{-2}$ where σ is the interfacial tension, ν the viscosity of the fluid between the interfaces and A the Hamaker constant. They concluded that the droplets have to be in close contact with the pattern for a time in the order of tenths of milliseconds to milliseconds and increasing the droplet velocity will decrease this time of contact ultimately resulting in coalescence prevention.

Geometrically mediated droplet coalescence

Tan et al.[17] demonstrated fusion of microdroplets by mediating them geometrically at a trifurcating junction and allowing periodically formed, equally spaced out emulsion droplets to redistribute and fuse consistently.

They varied the drainage rate, longer hydrodynamic trapping time to increase the number of fused droplets rearranged the original droplet formation pattern accordingly. They distributed the formation pattern of individual small droplets evenly in a straight channel and thus can enter the trifurcating junction and form a pattern of fused doublets, triplets, and etc.

Medial fusion was observed when the droplets are perfectly aligned to the middle of

Figure 2.18. Geometry of the droplet fusion device. (Left) Droplets are created at the generation site using the flow focusing geometry. (Right) Droplets then travel down stream to the junction where they fuse according to the designed geometric ratios. Q_d is the sum of the upper fluid drainage rate and the bottom fluid drainage rate ([17])

Figure 2.19. Three types of fusion events were observed in the microfluidic device. The arrow indicates the traveling direction of the droplet. Medial fusion and lateral fusion differs in the positions of the droplets during fusion. During induced fusion the channel inlet deforms the droplet causing the coalescence of the subsequent drop. Shown in the last row is the sequential fusion of multiple droplets. Up to six droplets have been shown to fuse with this process ([17])

the junction before fusion and during fusion; the lateral fusion occurred when one droplet is in contact with the side of the other droplet but not aligned center to center. The induced fusion occurred when the deformation of the front droplet initiates the fusion of the following droplet.

They observed that, when droplets fuse the shape of droplets are temporarily deformed then restored which characterize the completion of fusion. The fusion of two droplets containing the same fluids was completed in approximately 16 ms. They reported that the time scale for their finding is different from the classical droplet coalescence by film drainage in which the film drainage time is on the scale of seconds.

Bremond et al.[58] proposed that decompressing emulsion droplets mechanism for the coalescence of droplets in microchannels. [59] reported merging two droplets with the use of pillars in microchannels. Merging reagents into the droplets moving in the mainchannel at a T-junction experiences problems like synchronization of droplet arrival, contamination of the injecting stream and reliable merging only in a narrow range of flow rates ([14, 42, 60]). Replacing single T-junction with multi-junction eliminates the need for synchronisation ([13]), but has higher fabrication cost due to the insertion of hydrophilic side channels separately. Injecting reagents alternatively from two side branches of double T-junctions increases the synchronization frequency in a wide range of flow rates but merging is not guaranteed to 100% at all flow rate conditions ([61]). Therefore, there is a need for designs which add reagents to droplets reliably and yet need to be simple for design and fabrication.

2.4. Droplet Micromixing

2.4.1. Introduction

In microscale, momentum, mass and energy transport experience laminar and Stokes flow conditions (i.e. low-Reynolds number, $Re < 1$) ([62, 31]). Unavailability of turbulent conditions make mixing dependent on the diffusive properties of the species involved in process ([45]). Mixing is one of the key operations in any chemical based analytical method. It is required for carrying out and studying the kinetics of biological and chemical reactions. In micro-total-analysis systems (μ -TAS), species involved in the analysis are macromolecules and biological species with low mass diffusivities and hence have long mixing time in laminar flow conditions. Chaotic advection has been proven as a good candidate for mixing high

viscous fluid laminar fluids in macroscale dimensions and becoming popular in mixing low viscous fluids in microscale.

From the available literature ([63, 64, 46]), it is well understood that chaotic advection can be produced whenever the kinematic equations of motion for passively advected particles give rise to a nonintegrable dynamical system. The mathematical model of mixing, which is equivalently called as stirring, arises by considering particles that are advected passively by a prescribed or evolving velocity field. Understanding the passive advection of particles in the complex laminar flow is considered as a useful first step in describing the mixing process in chaotic advection driven microscale mixing. Clever channel modifications have been implemented to improve the chaotic advection by promoting internal circulation of liquids in the vortices in droplets and slug. When a droplet moves through a straight microchannel, recirculating flow of equal size is generated in each half of the droplet ([23]). Fluids within each half of the droplet are mixed, but the two halves remain unmixed and separated from each other. Confocal μ -PIV (Micro-particle image velocimetry) has been used to visualise the vortices to understand the complex three-dimensional movements inside the slugs and droplets, which are thought to be the mechanism behind the enhanced mass transfer ([65]). They observed a complex three dimensional flows near the boundaries and a symmetrical flow characteristic to the channel center line. The recirculation zone divides the slug into two halves and mass transfer across the channel centerline is limited to diffusion ([66]).

To enhance internal mixing within droplets, modifications in the channel geometry, by employing turns and bends, are used to create chaotic advection to fold and stretch the content of the droplet ([20, 39]). As the droplet traverses through a curved channel, the two halves of the droplet experience unequal recirculating flows. One half of the droplet is exposed to the inner arc of the winding channel, a shorter channel section, and thus a small recirculating flow is generated compared to the other half of the droplet which is exposed to a longer channel section. The irregular motion along the walls promoted chaos and crossing of fluid streams since the vortexes of each half are assymetrical. The influence of curvature in a two-phase flow is completely different from single-phase flow which generates the so called Dean flow ([67]). In two-phase flow the effect of curvature is restricted to changing the size of the two vortices. The droplets achieve an alternating assymetric flow pattern through the serpentine microchannels. The sharp turns also helps to reorient the droplet so that it becomes thoroughly mixed as it goes through a series of stretching, reorientation, and folding

Figure 2.20. *Schematic illustration of the approach characterized to form plugs of multiple aqueous solutions by injecting them into a stream of water-immiscible fluorinated fluid. Droplets were mixed rapidly by recirculation shown by white arrows ([18])*

([68]).

2.5. Segmented Flow Micromixers

Dispersion is a problem associated with pressure-driven laminar flow in microfluidic channels, due to parabolic velocity profile, and move the reagents at different velocities across the width of the channel. Droplet and segmented flow micromixers have been realized to avoid the dispersion problem by localizing the reagents within droplets surrounded by an immiscible fluid. Reagents no longer disperse along the whole length of the channel; rather dispersion is confined to the volume of the plug. The question is to determine the moment when the reagents are perfectly mixed within the droplet. [18] have developed a technique where multiple fluids are mixed by recirculation inside a confined liquid droplets that are dispersed in an immiscible liquid (Figs. 2.20 , 2.21). They reported the experimental characterization of a simple method for rapid formation of droplets, or plugs, of multiple aqueous reagents without bringing reagents into contact prior to mixing. They used straight channels and demonstrated that, when travelling through straight microchannels, mixing within plugs by steady recirculating flow is highly sensitive to the initial distribution of the aqueous reagents established by the eddy flow at the tip of the forming plug. They also showed how plugs with proper distribution of the aqueous reagents could be formed in order to achieve optimal mixing of the reagents in the system.

Gunther et al.[19, 41] have shown that liquids can be mixed by recirculation that is associated with the introduction of gas phase that forms a segmented gas-liquid slug flow

Figure 2.21. *Schematic comparison of a reaction $A+B$ conducted in a standard pressure-driven microfluidic system device (a), and the meandering droplet based microfluidic mixer. When three streams enter the channel with a flowing immiscible liquid, they form droplets ([18])*

as shown in Fig. 2.22. They found that the length required to mix miscible liquids linearly depend on the slug or droplet length and weakly depend on Peclet number of the flow. For gas-liquid flow with a uniform slug length on the order of the microchannel width, the required mixing length is approximately 30 times the channel width. In segmented gas-liquid flows, the discontinuous phase is provided by gas bubbles that reduce the axial dispersion in the liquid phase.

Decreased dispersion, i.e a sharpened Residence Time Distribution (RTD), is important for microfluidic networks since it increases the yield of chemical reactions conducted in the microchannel.

In the case of particle synthesis, the narrow RTD is translated to a visible product quality, the particle size distribution (PSD). Segmented gas-liquid flows were shown to greatly reduce axial dispersion and micro droplet flows are practically dispersion free. Traschel et al.[69] developed an integrated technique that allowed the injection of 100nl of tracer volume into a segmented gas-liquid flow and measurement of the RTD in a 400 μm wide and 1200 mm long microchannel network. This technique can be applied to more complex gas-liquid flow configurations, e.g. to structured microchannel networks.

Garstecki et al.[9] demonstrated the segmented gas liquid flow for micromixing that is sufficiently simple that it can be used in portable microfluidic devices as shown in Figs. 2.23 and 2.24. They illustrated the use of the micromixer by incorporating it into an elementary, portable microfluidic system that includes sample introduction, sample filtration, and valv-

Figure 2.22. *Representative fluorescence micrographs showing liquid segments (top), the corresponding velocity vector fields (center), and streamline contours (bottom) from PIV measurements in straight channel (left) and meandering channel (right) ([19])*

ing. They designed the structure of the channels ensure mixing of the laminar streams by interaction with bubbles of gas introduced into the channels.

Song et al.[20] described an experimental test of a simple argument that predicts the scaling of chaotic mixing in a droplet moving through a winding microfluidic channel as shown in Figs. 2.25, 2.26. Previously, scaling arguments for chaotic mixing have been described for a flow that reduces striation length by stretching, folding, and reorienting the fluid in a manner similar to that of the bakers transformation. They observed the flow patterns experimentally inside the droplets and found that they resembled the bakers transformation. Therefore, they argued that the ideas described in the literature could be applied to mixing in droplets to obtain the scaling argument for the dependence of the mixing time, $t \sim (aw/v)\log(Pe)$, where $w[m]$ is the cross sectional dimension of the microchannel, a is the dimensionless length of the plug measured relative to w , $v[ms^{-1}]$ is the flow velocity, Pe is the Peclet number ($Pe = wv/D_{mol}$), and $D_{mol}[m^2s^{-1}]$ is the diffusion coefficient of the reagent being mixed. Under favourable conditions, they demonstrated sub millisecond mixing.

Liau et al.[21] reported a droplet based micromixer that induces chaotic mixing of crowded solutions in milliseconds due to protrusions of the microchannel walls that generate oscillating interfacial shear within the droplets as shown in Fig. 2.27 .

The mechanism for the mixing inside the dilute aqueous plugs is achieved by the alter-

Figure 2.23. *Micrographs of the micromixers. (a) Laminar flow without bubbles (b) Plugs flowing through the networks. In the first branching section, there are two bubbles in the left arm and one bubble in the right arm. The lower resistance in the right arm redirects a portion of the black liquid into the right (originally clear) channel. The dashed lines mark the positions at which we measured the intensity profile across the width of the channel ([9])*

Figure 2.24. *Pictures and micrographs of the portable microfluidic device ([9])*

Figure 2.25. *Schematic of a fluid element undergoing stretching, folding and reorientation, characteristics of the bakers transformation (top). Stretching and folding, as defined here, without reorientation (bottom) does not lead to decrease of the striation thickness, demonstrating the critical nature of the reorientation step ([20])*

Figure 2.26. *The bakers transformation in plugs moving through a microfluidic channel. (a) Schematic illustrating the principle: Straight portions of the channel perform stretching and folding, and turns allow for reorientation. (b) Mixing as represented by a scheme of recirculating flow in plugs moving through smooth turns (i) and sharp turns (ii). (c) Microphotographs of the microfluidic network in which flow patterns inside plugs in different positions in the microchannel demonstrate flow patterns reminiscent of the bakers transformation ([20])*

Figure 2.27. *Schematic of the bumpy serpentine mixer. Two streams of crowded solutions (CS) containing reactants and X and Y separated by a third stream of crowded solution intersect with two oil streams to form droplets suspended in oil (plugs). The plugs then proceed through n cycles of bumpy serpentine channel until the plug contents are fully mixed ([21])*

nation between two asymmetric flow patterns within the plugs, induced as the plug traverses each half-cycle of the serpentine channel.

Tang et al.[22] reported a microfluidic system that relies on chaotic advection to rapidly mix crowded biological solutions that have been isolated in droplets (slugs) as shown in Fig. 2.28. They forced the slugs through a pillar matrix to stretch and fold them repeatedly, thus creating fluid flows that rapidly mix the slug contents in the order of milliseconds at Reynolds number in the order of 10^{-3} . They suggested that the pillars generated a significant amount of interfacial stresses on the slugs and in the process induced rapid mixing.

Micromixing in continuous phase flows and droplet microfluidics have been comprehensively reviewed by ([70, 71, 39]). Therefore, the next section is devoted to the works related to mathematical modeling of droplet micromixing.

Bejan et al.[72] constructed a theory of geometry generation (selection, evolution) during molten droplet impact relying on the constructal law of maximization of flow access. They showed that immediately after impact the liquid spreads inviscidly as a ring with a radial velocity that scales with the initial impact velocity. And through the theoretical model, they showed that if the initial droplet is small and slow enough, the splat comes to rest (dies) viscously, as a disc. If the droplet is large and fast enough, the ring splashes and is continued outward by needles that grow radially until they are arrested by viscous effects. The results are applicable where droplet impact behavior spells success or failure, especially in

Figure 2.28. *Schematic and microphotograph of the (a) straight and (b) serpentine channels with pillars ([22])*

new technologies based on using small scale droplets.

2.6. Mathematical Modeling

Handique and Burns[23] proposed a mathematical model to estimate mixing time for slugs having axially arranged reactants in a straight slit microchannel (Fig. 2.29). They computed the velocity profile inside the droplet moving in slit through superimposing the continuous flow velocity on the reference frame of droplet. The calculated velocity profile as,

$$V_{rel}(y) = 0.5V_d \left(1 - 3 \left(\frac{y}{d/2} \right)^2 \right) \quad (2.1)$$

where the V_{rel} is the velocity of a streamline at a distance y from the centerline axis. They found that the relative velocity of the streamline V_{rel} drops to zero at $y = y_s = 0.577(d/2)$ (stagnation line) and a liquid element moving on its streamline at position y appears at a corresponding streamline at position y^* when it reaches the end of the drop. They related the streamline pairs (y and y^*) by performing a volumetric flow balance around the stagnation line and are given by the following expression,

Figure 2.29. *a) A discrete drop placed in a slit-type microchannel, where the width of the channel is very large compared to the depth of the channel. (b) Recirculation streamlines in a drop moving at a constant uniform velocity in a slit type channel. The frame of reference is moving at the average velocity of the drop. Liquid in the middle of the drop moves to the leading end of the drop and then towards the channel wall. At the receding end of the drop, liquid moves from the wall towards the center of the drop ([23]).*

$$\frac{y^*}{d/2} = -\frac{y}{d} + \frac{\left[4 - 3\left(\frac{2y}{d}\right)^2\right]^{\frac{1}{2}}}{2} \quad (2.2)$$

In order to estimate the concentration profile of the solute, the following coupled convective and diffusive solute transport equation was solved in conjunction with the velocity profile given in equation 2.1.

$$\frac{\partial c}{\partial t} + V_{rel} \frac{\partial c}{\partial x} = D \frac{\partial^2 c}{\partial x^2} + D \frac{\partial^2 c}{\partial y^2} \quad (2.3)$$

When the drop is displaced rapidly from its initial position, recirculation is created in the liquid. Because of the non-uniform velocity profile across the channel depth, the fluid elements are non-uniformly displaced with respect to each other, thereby increasing the intermaterial area. The distribution of liquid was determined as a function of the drop lengths moved and is plotted in figure 2.30.

They performed detailed modelling calculations exploring the effect of velocity, channel dimension and solute diffusivity on the mixing of solutes in a discrete drop for two limiting cases: when convection dominates over diffusion and when diffusion dominates over convec-

Figure 2.30. *Effect of streamlines on the distribution of immiscible liquids. (a) Two equal-sized drops are placed end-to-end at $t = 0$, (b) The combined drop is moved by a drop length, causing the receding drop to interlayer between the leading drop. Further interlayering is caused after the drop is moved by (c) two drop lengths, (d) three drop lengths and (e) four drop lengths ([23])*

tion. Based on these analyses, they outlined a mixing strategy. The technique for calculating the mixing time involves calculating the critical interlayering velocity (V_c), microchannel (depth, d) and drop length (L). Furthermore, to mix a solution containing multiple solutes with widely varying diffusivities, the critical interlayering velocity is calculated based on the diffusivity of the smallest molecule and the post-interlayering mixing time estimated based on the diffusivity of the largest molecule.

Che et al.[24] reported an analytical model for a liquid plug moving in curved microchannels as shown in Fig. 2.31. They showed that for a plug moving in a microchannel with low curvature, the vortex centre shifts towards the wall of the microchannel when the

Figure 2.31. *Schematic of the flow in a curved microchannel ([24])*

Figure 2.32. *Snapshots of mixing patterns taken at the non-dimensional times from left to right $t^* = 0, 0.42, 0.90, 1.32, 1.80, 2.22, 2.64$ ([25])*

plug length is small. But for a given plug length, the centre of the vortex moves toward the direction of the inner wall as the channel curvature is increased. They suggested that the vortex patterns of the plug can be controlled by designing the channel geometry appropriately.

2.7. Numerical Simulation

Muradoglu and Stone [25] studied chaotic mixing in a droplet moving through a serpentine microchannel computationally (Fig. 2.32) in a two-dimensional setting using a direct numerical simulation within the framework of a finite-volume (FV) /front tracking (FT) method. They used tracer particles to visualize the mixing patterns and studied the mixing and examined the effects of the various non-dimensional parameters on mixing. They found that the best mixing is observed when the drop size is comparable with the channel width, the capillary number strongly influences the mixing process and smaller the capillary number, corresponding to smaller drop deformation, the better the mixing. They also reported that viscosity ratio of the drop phase to ambient fluid has strong influence on quality of mixing. In contrary to the intuition, they found that the Reynolds number has no significant influence on mixing but smaller the Reynolds number, the better quality of mixing.

Muradoglu and Stone[26] numerically studied the axial dispersion of a tracer in a two-dimensional gas-liquid flow as shown in Figure 2.33. The axial dispersion in gas-liquid segmented flow is a result of mass transfer from the bulk fluid to the slow moving film region by a combined effect of molecular diffusion and convection due to recirculation in the liquid slugs. Although axial dispersion is much reduced in the segmented gas-liquid flow compared to an

Figure 2.33. *Schematic illustrations of a two-bubble system (top) and a bubble train ([26])*

equivalent single-phase system, because of the nearly stagnant film region there may still be significant back mixing especially in the case of small Peclet numbers and for larger capillary numbers where films are thicker. They used a large number of passive tracer particles to visualize and quantify mixing in the continuous fluid and the molecular mixing is modelled by a random walk of tracer particles, which are advected with the local fluid velocity interpolated from the Eulerian grid. They reported that, the axial dispersion is essentially controlled by convection through the liquid films between the gas bubble and channel walls and it becomes independent of the Peclet number in the limit as Pe tends to zero. On the other hand, when the Peclet number is sufficiently large, the axial dispersion is mainly controlled by the molecular diffusion of the tracer across the boundary between the recirculating bulk region and the film region.

Stone and Stone [27] described a backtrace imaging method that can render uniform cross sections of the droplet at arbitrary times in the mixing process and introduced a scalar measure of mixing that can be applied directly to a colour-labelled two-or three-dimensional grid (Figures 2.34, and 2.35). The actual flow field inside a droplet carried by a second immiscible liquid down a serpentine channel with a rectangular cross section is very complicated, especially when the diameter of the undeformed droplet is comparable to or larger than the channel width. To construct a simple model of the flow internal to the droplet, they chose to focus on the most basic features of the serpentine channel mixer: curves and

Figure 2.34. *A comparison of the serpentine channel mixer with analytical model of the droplet mixer. Though the model does not represent the complex flow in the serpentine mixer, it mimics the mixers four-step periodic flow pattern. (a) The serpentine channel mixer broken into a periodic sequence of four segments: a curve, a straight segment, a reverse curve, and a second straight segment. (b) The sequence of four external flows applied to a spherical droplet in the model mixer, illustrated in the reference frame of the droplet: a superposition of uniform and shear flow, a uniform flow, a superposition of uniform and opposite-signed shear flow, and a uniform flow. Transitional flows are neglected ([27])*

straight segments of varying lengths; noting that it has been observed in experiments that the mixing characteristics are not significantly affected, for example, by whether a turn is sharp or smooth. The idealized mixer consists of a periodic sequence of four channel segments connected in the following order: a curve, a straight segment, a reverse curve, and another straight segment.

Although it is common to report suitable chosen Poincare sections to characterize the degree of mixing caused by a flow, such approaches give no indication of the time evolution of the actual mixing process. Hence, they developed a backtrace imaging method to render uniformly sampled cross sections and three-dimensional grids at arbitrary times in the mixing process. They found that the best mixing occurs when the viscosity ratio between the fluid inside the droplet and the surrounding fluid is small.

2.8. Dynamical Systems and Deterministic Chaos

In any fluid system, the motion of a fluid particle is governed by Navier-stokes equations and the equation of continuity, with constraints imposed by boundary. These equations together with the appropriate boundary conditions constitutes a dynamical system. The solution of

Figure 2.35. *A step-by-step illustration of the backtrace imaging procedure, which makes it possible to image any configuration of points in the droplet directly at an arbitrary time in the mixing process ([27])*

these equations gives the time evolution of the system. It is very important to characterize a dynamical system by a few meaningful numbers, called invariants of the system. These invariants help in classifying or identifying the physical source of the observations and provide means to make models for prediction and control of the nonlinear system.

The irregular, chaotic motion that is generated by nonlinear systems whose dynamical laws uniquely determine the time evolution of a state of the system from a knowledge of its history is called deterministic chaos. Nonlinearity is a necessary but not a sufficient condition for a dynamical system to exhibit chaos. The observed chaotic behaviour in time is neither due to external sources of noise nor to an infinite number of degrees of freedom. The reason for irregular motion is the property of the nonlinear system of separating initially close trajectories exponentially fast in a bounded region of space. It is therefore practically impossible to predict the long-time behaviour of chaotic dynamical systems, because in practice the initial conditions can only be fixed with some finite accuracy.

2.9. Chaotic Advection

The topic of chaotic mixing in a liquid droplet in a low- Reynolds number flow was first studied theoretically by Bajer and Moffat ([73]) and then by Stone [74] and Kroujiline[75].

These studies make clear that the three-dimensional flow in a spherical droplet may exhibit chaotic streamlines in steady state conditions and other driving forces for fluid motion, either steady or unsteady, are capable of producing three-dimensional mixing flows inside a droplet. When a particle moves with the fluid, we speak of advection, sometimes passive advection to emphasize that the particle is so light and inert that it can do nothing but follow the fluid, instantaneously adjusting its own velocity to that of the ambient flow. We may write

$$V_{particle} = V_{fluid} \quad (2.4)$$

as the formal statement of passive advection. In particular, the kinematics of the fluid itself is such that each fluid particle undergoes passive advection.

The particle velocity, $V_{particle}$, is, of course, given by the rate of change of its position:

$$V_{particle} = \left(\frac{dx}{dt}, \frac{dy}{dt}, \frac{dz}{dt} \right) \quad (2.5)$$

where (x, y, z) is the position vector of the particle, here written in ordinary Cartesian coordinates.

The fluid velocity, which involve the solution of some set of partial differential equations, such as the Euler equations, the NavierStokes equations, or the Stokes equations.

$$V_{fluid} = [u(x, y, z, t), v(x, y, z, t), w(x, y, z, t)] \quad (2.6)$$

The condition that particle velocity equals fluid velocity then leads to a system of ordinary differential equations (ODEs), called, the advection equations:

$$\frac{dx}{dt} = u(x, y, z, t), \frac{dy}{dt} = v(x, y, z, t), \frac{dz}{dt} = w(x, y, z, t) \quad (2.7)$$

which are the so-called advection equations and provide a Lagrangian description of the fluid motion. The above two-dimensional advection equations are integrable if the flow is steady, while for unsteady flow they may be non-integrable. Integrable solutions of the advection equations lead to regular advection, and the non-integrable cases are characterized by chaotic advection which can be described as particle motion sensitive to initial conditions, i.e., initially nearby trajectories diverge at an exponential rate. To produce chaotic advection, the Eulerian velocity field in the advection equations is not necessarily very complicated

(turbulent); the Lagrangian particle trajectories can become chaotic even when the Eulerian velocity at any given fixed point in space is periodic in time, e.g. the blinking vortex (BV) flow.

The key for effective mixing lies in producing stretching and folding; stretching and folding may be roughly equated with chaos. The simplest case corresponds to two dimensions. If the velocity field is steady, the mixing is poor and stretching for long times is linear, as in the case of a simple shear flow; *i.e.* the stretching rate of line elements decays as $1/t$. It is relatively straight forward to produce flow fields that can generate stretching and folding and hence chaos. In rough terms, a necessary condition for chaos is the crossing of streamlines. That is, two successive streamline portraits, say at t and $t + \Delta t$ for time periodic two-dimensional flows, or at z and $z + \Delta z$ for spatially periodic flows, when superimposed, should show intersecting streamlines when projected onto the (x, y) -plane. In two-dimensional systems this can be achieved by the time modulation of the flow field, for example, by motions of boundaries or time periodic changes in geometry.

2.9.1. Characterization of chaotic mixing

Mixing in droplets have been described due to the mechanism that reduces striation length by stretching, folding, and reorienting the fluid which can be described by certain transformations. The important and most widely used transformations are described in detail in this section.

2.9.2. Bernoulli transformations

A measure-preserving transformation is called *Bernoulli* if it is isomorphic to a Bernoulli shift ([28]). The Bernoulli shift is the paradigm for *deterministic chaos*. In general the following implications holds:

$$Bernoulli \rightarrow mixing \rightarrow ergodic,$$

and the direction of the arrows cannot be reversed. Thus, Bernoulli is the most desired property for mixing and the baker's transformation is the best mixing transformation (Proof given in ([28])).

2.9.3. Baker's Transformation

The baker's map is probably one of the most famous mathematical transformations in the theory of conservative dynamical systems. The baker's map is obtained by iterating the elementary transformation S defined on the unit square, with periodic boundary conditions.

$$S(x, y) = \begin{cases} (2x, \frac{1}{2}y) \bmod 1 & \text{if } 0 < x < \frac{1}{2} \\ (2x, \frac{1}{2}y + 1) \bmod 1 & \text{if } \frac{1}{2} < x < 1. \end{cases}$$

The specific behavior of the map appears when considering how the upper and lower half of the square are mapped when the transformation S is repeatedly applied. Successively squeezed in the y direction while stretched in the x direction, cutting in two and then stacking the right half above the left, causes what is initially a two-strip domain to become four strips after one iteration as shown in Fig.2.36. We can get an idea of the bakers transformation applied to the black and white regions shown in Fig. 2.36. Let B denote the region of black material and W the region of white material. The area of B , denote $\mu(B)$, is equal to $1/2$. The area of W , denoted $\mu(W)$, is also equal to $1/2$. After n advection cycles, we have 2^{n-1} black strips, each of length 1 and width $1/2^{n+1}$. Mathematically, $S^n(B) \cap W$ denotes the black material that is in the region originally occupied entirely by white material. Hence, we have $\mu(S^n(B) \cap W) = \frac{1}{4}$, and therefore

$$\lim_{n \rightarrow \infty} \mu(S^n(B) \cap W) = \mu(B)\mu(W). \quad (2.8)$$

The bakers map is a mixing transformation since, in the limit $n \rightarrow \infty$, the “unit square becomes completely filled with an infinite number of alternating red and yellow lines” and thus any infinitesimal region of the square contains the same proportion of red and yellow.

2.9.4. Statistical Characterization of Chaotic Mixing

To calculate the mixing efficiency, statistical tools such as ‘Variance Index (I_{var})’, ‘Shannon entropy Index (I_S)’ and ‘Complete Spatial Randomness (CSR)’ are widely used. Variance as a mixing measure has been used widely to characterize the chaotic mixing along with Shannon entropy index ([76]).

Figure 2.36. (a) One iteration of the baker's transformation on the unit square, (i) at the beginning the upper half of the square is black and the lower half is white. The square is squeezed, stretched, cut, and re-stacked. (b) The second iteration of the baker's transformation. (c) The fourth iteration of the baker's transformation. Note how quickly the black and white material is mixed. Also note that there is no loss of material from the original domain (the unit square) ([28])

Variance Index

Variance of the particle counts in the bins is calculated by dividing the whole flow domain in to $n \times m$ grid of equal-size bins. The variance σ^2 is calculated as follows,

$$\sigma^2 = \frac{1}{M} \sum_{j=1}^M (c_j - \bar{c})^2 \quad (2.9)$$

where, M is the number of bins and c_j is the number of particles in bin j , $\bar{c}$ is the average number of particles per bin, $\bar{c} = N/M$, with N the total number of particles in the calculation. The variance decreases with number of periods as mixing improves, and particles are distributed in the computational domain. Variance Index, which varies from unity to zero, is defined as follows

$$I_{var} = \frac{\sigma^2}{\sigma_0^2}, \quad (2.10)$$

where, σ_0^2 denotes the most segregated state which can be deduced from equation (2.9) as follows, when $M \gg 1$. While calculating variance for particle distribution using bin counts two simple limiting cases exist: the first is a completely segregated mixture; the second limiting case is an even distribution. For the first case, for a given bin size this occurs when all Lagrangian particles reside in a single bin. Therefore, using a subscript 0 to denote this most segregated state, the segregated variance is

$$\sigma_0^2 = \frac{1}{M} \left\{ (N - \bar{c})^2 + (M - 1) (\bar{c}^2) \right\} \quad (2.11)$$

and we know, $\bar{c} = N/M$. Therefore, replacing $\bar{c}$ with (N/M) ,

$$\sigma_0^2 = \frac{1}{M} \left\{ (N - (N/M))^2 + (M - 1) (N/M^2) \right\} \quad (2.12)$$

Expanding the first term inside the bracket,

$$\sigma_0^2 = \frac{1}{M} \left\{ ((N)^2 + (N/M)^2 - 2N^2/M) + (M - 1) (N/M^2) \right\} \quad (2.13)$$

Simplifying the terms,

$$\sigma_0^2 = N^2 \{(M-1)/M^2\} \quad (2.14)$$

For the case where $M \gg 1$, the above equation can be simplified to

$$\sigma_0^2 = N^2/M \quad (2.15)$$

The second limiting case, where the particles are evenly distributed, using the subscript ‘e’, has the variance $\sigma_e^2 = 0$. This index ranging from unity (maximum segregation) to zero (even distribution) allows easy comparison of calculations using various boundary conditions in this investigation.

Shannon Entropy

Shannon entropy S , which originated in information theory and statistical mechanics, is another measure used to analyse chaotic mixing widely and is defined as

$$S = - \sum_{j=1}^M p_j \ln p_j, \quad (2.16)$$

where, p_j is the probability that a particle will lie in bin j . With Lagrangian Particle Method, the probability is taken as the particle count divided by the total number of points, or $p_j = \frac{c_j}{N}$. Shannon Entropy Index I_S was calculated by using the following relationship,

$$I_S = 1 - \frac{S}{S_e} \quad (2.17)$$

This form of the normalization makes it easy to see the details as a mixture approaches uniformity, by plotting I_S on a logarithmic scale.

Complete Spatial Randomness

Complete Spatial Randomness (CSR), which is the even distribution of material points through out the flow domain, is taken as the measure of complete mixing. This is an ideal state for a physical mixture. However, in Lagrangian Particle tracking method, repeated iterations of a globally chaotic flow do not result in an even distribution of the particles, but rather one in which each particle is equally likely to lie in any bin.

According to the binomial probability mass function (PMF) of the bin counts, for $M \gg 1$ and $N \gg 1$ the binomial PMF asymptotes to the Poisson PMF,

$$p(c) = \left(\frac{N}{M}\right)^c \frac{e^{-\frac{N}{M}}}{c!} \quad (2.18)$$

with a variance of $\sigma_{CSR}^2 = N/M$ and this gives the CSR limit for variance index $I_{var,CSR} = 1/N$ and for the entropy index the limit is $I_{S,CSR} = M/2N \ln(M)$. Once a random distribution of particles throughout the flow is achieved, the limiting values of variance and Shannon entropy index are reached. CSR is a fundamental numerical limit for any Lagrangian particle calculation and no further distribution of particle can be attained.

2.10. Summary and Motivation

From the literature review, we can understand that the process of droplet formation in microfluidic channels has received significant attention over the last decade. Significant efforts have been targeted towards developing a droplet-based microfluidic system and understanding the droplet formation process and the physics behind it. It is observed that most of the researchers have been focused on the effect of operating variables such as capillary number, flow rates and their ratio, viscosity ratio etc. However, the underlying physics of the droplet formation in terms of evolution of pressures in both the continuous phase and dispersed phase has received less attention over the years and is not understood clearly.

As a next step in the analysis, droplets containing the sample in the microfluidic device need to be mixed with reagents for further analysis; adding the reagents to the droplets is a fundamental operation as it allows the precise mixing of reagents at well-defined points in space and time. Adding precise amount of reagents to a droplets poses difficulties and various schemes for reagent addition have been investigated by researchers as seen in section 2.3.2. However, many of the available designs use complicated structures and fabrication methods and it is necessary to find a simple design to reliably add reagents into droplets.

After the addition of reagents to the droplets, the contents need to be mixed as fast as possible to reduce the process time to have a high throughput. Microchannel designs adding reagents to the droplets and promoting droplet micromixing have been reported but the strategy behind the designs are basically intuitive and there is a lack of design methods based on first principles.

Therefore, this study concentrates on the mechanism of droplet formation in a microfluidic T-junction and develops a new understanding, in terms of pressure fluctuations in the continuous phase and the dispersed phase, based on the observations made in the experimental and numerical investigation. Secondly, a microchannel design for reliably merging reagents to droplets at a T-junction which can also promote mixing is developed and the physics of reliable merging is understood through numerical investigation. Thirdly, a simple method for evaluating and predicting chaotic advection in a microchannel is developed through a numerical investigation.

3. Microfabrication Procedures and Experimental Setup

3.1. Introduction

This chapter presents the details of the microfabrication of microfluidic channels through softlithography technique using PDMS(polydimethylsiloxane) in section 3.1.1. In section 3.2 the designs and dimensions of the microfluidic channels used in this study are delineated. Section 3.4 presents the imaging setup and experimentation techniques used in this research.

3.1.1. Softlithography

Initially microfluidic systems used silicon and glass to fabricate the devices. But for analyses of biological samples in water, devices fabricated in glass and silicon are usually unnecessary or inappropriate. Silicon, in particular, is expensive, and opaque to visible and ultraviolet light, so cannot be used with conventional optical methods of detection. Therefore, much of the exploratory research in microfluidic systems has been carried out in a polymer — polydimethylsiloxane or PDMS — though polycarbonate or polyolefin can also be used. The ease with which new concepts can be tested in PDMS has made it the key material for exploratory research and research engineering at the early stages of development ([77]).

3.1.2. PDMS

The microfluidic channels used in for this research were fabricated using PDMS through “softlithography” which refers to a set of methods for fabricating or replicating structures using “elastomeric stamps, molds, and conformable photomasks”. The main advantages are that devices can be produced very quickly, inexpensively and with a high throughput. This fabrication technique can be performed in an normal laboratory environment without an

expensive clean room, it is not diffraction limited, many of the processes are additive and the waste of materials is minimized.

PDMS is particularly known for its unusual rheological (or flow) properties. It is optically clear, and is generally considered to be inert, non-toxic and non-flammable. PDMS is durable elastomer, deformable, homogeneous, and isotropic. Its surface is low in interfacial free energy and chemically inert. The surface properties can be modified by treatment with plasma followed by the formation of self-assembled monolayers (SAMs) to give appropriate interfacial interactions with materials. In addition, it is inexpensive, flexible, optically transparent to wavelengths greater than 230nm, impermeable to water. Due to the CH₃ groups the PDMS surface is very hydrophobic. Treating the PDMS surface with oxygen plasma, the hydrophobic surface will become hydrophilic. If the PDMS is left on air after the oxygen plasma treatment, the hydrophilic surface tends to revert to hydrophobic in approximately 30 minutes.

3.2. Procedure of Softlithography

3.2.1. Photo-mask Fabrication

A photo-mask is a stencil, containing the microchannel designs, used to generate the microchannel patterns repeatedly on a resist-coated wafer. The microchannel designs were drafted using a computer-aided-design (CAD) program. The CAD-generated patterns were then sent for mask printing on a plastic transparency using commercially available services.

3.2.2. Master Mold Fabrication

Master mold is the pattern of microchannel designs on a silicon wafer, which is usually a positive relief of photoresist. To fabricate the master, the photolithography steps involved are: (i) Wafer preparation; (ii) Photoresist coating; (iii) Soft baking; (iv) Photoresist developing. A detailed process flow is shown in Fig. 3.1.

Wafer Preparation

Before the photoresist was coated onto the wafer, the wafer was cleaned to remove any contaminants such as

Process Flow

dust, solvent stains, etc. To clean the wafer, the wafer was rinsed with piranha etch — a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) — which cleans organic residues off the wafer, followed by rinsing with DI (de-ionized) water. Next, the wafer was baked in an oven at 200°C for 30 minutes to evaporate any residue water on the wafer.

Photoresist coating

The photoresist used in the fabrication is negative SU-8, a photo-curable epoxy, which strengthens when exposed to UV radiation by forming cross-linkage of the main chains. For coating of the photoresist, Karl Suss RC5 Gyrset spin coater was used. SU-8 100 was poured on the wafer surface and then spun to ensure uniformity. There are two cycles in the spin coating process; spread cycle and spin cycle.

For spread cycle, the speed was ramped to 500 rpm at an acceleration of 100 rpm/seconds to spread the resist. The spread cycle was followed by a spin cycle. The rotation was ramped to the final speed at an acceleration of 300 rpm/second and held for a total of 30 seconds. To obtain a thickness of 100 μ m, the final speed of 3000 rpm was used.

Soft Baking

Soft baking was done to evaporate the solvent and densify the film. After spin coating, the resist usually contains up to 15% solvent and have stress build-up. To remove the remaining solvent, the SU-8 resist was baked on a level conventional hot plate. For a thickness of 100 μ m, the SU-8 resist was first baked at an initial bake of 65°C for 10 minutes, followed by a final baking at a temperature of 95°C for 30 minutes.

Wafer Exposure

For the exposure of the photoresist, OAI mask aligner Model 500 IR was used. The optimal exposure dose is dependent on the film thickness. For a resist thickness of 100 μ m, a dosage of around 600 mJ/cm² was applied to properly transfer the mask image onto the resist.

Post Exposure Baking

After the exposure of the SU-8 resist was complete, post baking was done which selectively cross-links the exposed portion of the film and to ensure that the reactions initiated during the exposure are completed. A temperature of 65°C was applied to the wafer for 3 minutes and followed by a second post bake temperature of 95°C for 10 minutes.

Photoresist Developing

Before the developing process, the wafer was allowed to cool down to prevent thermal pressure due to sudden change in temperature. Then, the whole wafer was immersed in MicroChem's SU-8 Developer for a duration of 10 minutes. The exposed portions of the photoresist are insoluble to the developer solution and therefore the unexposed portions of the photoresist were dissolved away by the developer, leaving the microchannel patterns on the wafer intact.

Wafer Cleaning

Once the master was completed, the surface was rinsed briefly with isopropyl alcohol (IPA) and was gently dried with a stream of air from the air gun.

3.2.3. PDMS Microchannel Fabrication

Once the master was completed, there are four steps involved in the PDMS microchannel fabrication. They are (i) PDMS mixture preparation; (ii) PDMS mixture curing; (iii) PDMS post processing and (iv) PDMS bonding.

PDMS Mixture Preparation

For the preparation of the PDMS mixture, liquid Dow Corning Sylgard PDMS pre-polymer was mixed with curing agent in a ratio of 1:10 (curing agent: base polymer). The amount of liquid PDMS depends on the size of the petri dish used and the desired chip thickness. Then, the PDMS mixture was stirred thoroughly and it was placed in a vacuum chamber to remove all the air bubbles. The mixture was poured onto the master once all the bubbles were cleared.

PDMS Mixture Curing

Before pouring the PDMS mixture, the master and the petri dish were cleaned from dust particles. IPA was applied to the wafer and it was blow dried using a nitrogen air gun. The master was then placed in the petri dish and the PDMS mixture was poured onto the petri dish-silicon wafer assembly. The PDMS mixture was poured slowly from the edge the wafer to prevent the creation of new air bubbles. Then the PDMS mixture was kept in oven at a temperature of 80°C for 2 hours.

PDMS Post Processing

After the mixture was fully cured the rubbery solid piece was cut into the desired shapes with the use of sharp razor blade. Then, the divided channels can now were removed from the petri dish by gently peeling it off from the master. The inlet and outlet holes were punched using 0.5mm Haris Uni-core Biopsy Punch.

PDMS Bonding

To complete the chip fabrication, the channels on the PDMS were closed by bonding it with another piece of plain PDMS after treating both surface with Oxygen plasma. To ensure good bonding, the surfaces of the PDMS were washed with IPA to make them and were dried using a nitrogen air gun. The cleaned PDMS were then placed in an oven at 65°C for 15 minutes to dry the surface completely. The parameters set for the system were: Base Pressure = 165 mTorr; Power= 100W; Oxygen concentration= 30%; Process duration = 40 seconds. Immediately after the plasma discharge, the two oxidized PDMS surfaces were brought into contact with each other to form a tight irreversible seal. Finally, the bonded chips were placed into an oven and are hold at a temperature of 80°C to strengthen the bonds between the PDMS surfaces and to remove the hydrophilic property caused by the oxygen plasma bombardment.

Figure 3.2. *Photograph of the PDMS chip fabricated through softlithography.*

3.3. Experimental setup

The experimental setup consists of four main components, a microfluidic device, reagents and solutions, a control system and an illumination and recording system as shown in Fig. 3.3.

Figure 3.3. *Experimental setup consisting of the microfluidic device, imaging setup (inverted microscope), syringe pumps and DVD recorder*

3.4. Imaging setup

The imaging setup consists of four main components: an illumination system, an optical system, a coupled charge device (CCD) camera and a control system. The control system consisting of a FLOW MANAGER System hub and its corresponding software FLOW MANAGER program is implemented in a personal computer. The PC based system thus control and synchronize all action related to illumination and image recording. The schematic diagram of the setup is shown in Fig. 3.4. A single mercury lamp acts the source of illumination for fluorescence imaging.

The optical system used was a Nikon inverted microscope (Model ECLIPSE TE2000-S) with a set of epi-fluorescent attachments. There are three optical elements in a filter cube: excitation filter, dichroic mirror and emission filter. Emission filters are used in both measurements to select a specific emission wavelength for the sample and to remove traces of excitation light. At the beginning of the experiment, the fluorescent was illuminated by a mercury lamp, an epi-fluorescent attachment of type Nikon B-21 was used (excitation filter for 450-490 nm, dichroic mirror for 505 nm and an emission filter for 520 nm) to take the measurement.

An interline transfer CCD camera (HISENSE MKII) was used for recording grayscale

Figure 3.4. *Schematic of the experimental setup for fluorescent imaging and μ -PIV measurements*

images and SONY DVD handy-cam was used for recording the magnified video from the eye-piece of the microscope. The resolution of the camera is $1344 \text{ pixels} \times 1024 \text{ pixels}$, with 12 bits grayscale. To ensure that the CCD camera is working at its optimum temperature of -15°C , a cooling system is integrated in the CCD camera. In the mode of double exposure in double frames, the camera can record two frames of the flow fields and then digitizes them in the same image buffer.

4. An investigation on the mechanism of droplet formation in a microfluidic T-junction

This work has been published in the journal of Microfluidics and Nanofluidics ([78]).

4.1. Introduction

From the available literature on the droplet formation process in a microchannel, as reviewed in the literature survey in section 2.2, it is clear that most of the researchers have been focused on the effect of operating variables such as capillary number, flow rates and their ratio, viscosity ratio etc. However, the understanding of the underlying physics of the droplet formation, in terms of evolution of pressures in both the continuous phase and dispersed phase, in the T-junction is not very clear. Garstecki et al.[49] postulated a mechanism based on the assumption that the pressure in the dispersed phase at the inlet remains constant throughout the droplet formation process (long-dashed line in Fig. 2.7(e)) and explained the process by the evolution of the continuous phase pressure (p_c). Though the assumption essentially reduced the complexity and enabled the process to be explained in simple terms, it didn't capture the real scenario. It is because, the dispersed (p_d) and the continuous (p_c) phase pressures are competing against each other at the junction to flow into the main channel; when the continuous phase pressure changes because of the blockage of the main channel by the dispersed phase, then the dispersed pressure will have to change accordingly, to remain in the competition and cannot remain constant.

While experimental work has helped to understand underlying physics, experiments at microscale are still difficult. For example, it is very challenging to measure pressure and velocity fields, droplet size, droplet deformation, break-up and coalescence at such small scales

([54]). Therefore, we employ a numerical method to study the dynamics of droplet break-up, especially the evolution of pressures of the continuous and dispersed phase to understand the underlying physics.

4.2. Geometry of the microchannel

Figure 4.1(a) shows the schematic of the three-dimensional rectangular microchannel and figure 4.1(b) shows the schematic of droplet generation in a two-dimensional microfluidic T-junction. The microchannel dimensions of the main channel are $100\ \mu\text{m}$ in height, $200\ \mu\text{m}$ in width and the length used is $1000\ \mu\text{m}$ and the side channel is $100\ \mu\text{m}$ in width and since the microchannel is planar the height is same as of the main channel (figures not drawn to scale). The dimensions of the channel were chosen keeping in mind the conditions for geometries that promote squeezing mechanism: (i) the width of the main channel should be greater than its height, and (ii) the width of the inlet channel should be at least equal to half the width of the main channel ([9]). The continuous phase (mineral oil) is pumped through the main channel and the dispersed phase (DI water) is pumped through the side channel. The notations p_d in the side channel and p_c in the main channel are for the positions at which the dispersed phase pressure and the continuous phase pressure are measured. These points are located at $50\ \mu\text{m}$ from both the upper and lower walls of the microchannel. (i.e. mid-plane of the three-dimensional channel).

4.3. Numerical simulation

4.3.1. Volume of Fluid (VOF) model

The transient three-dimensional numerical simulations of the multiphase flow of two immiscible fluids (oil and water) in microchannel with T-junction are performed using VOF model available in the commercial software FLUENT (Ansys Inc. USA). The VOF model can model two or more immiscible fluids by solving a single set of momentum equations and tracking the volume fraction of each of the fluids throughout the domain.

The VOF formulation relies on the fact that two or more fluids (or phases) are not interpenetrating. For each additional phase a variable is introduced: the volume fraction of the phase in the computational cell. In each control volume, the volume fractions of all

Figure 4.1. *A schematic illustration of the (a) three-dimensional microfluidic T-junction composed of planar rectangular channels with uniform height h . (b) two dimensional T-junction used in both experiment and computation*

phases sum to unity. The fields for all variables and properties are shared by the phases and represent volume-averaged values, as long as the volume fraction of each of the phases is known at each location. Thus the variables and properties in any given cell are either purely representative of one of the phases, or representative of a mixture of the phases, depending upon the volume fraction values. In other words, if the q th fluid's volume fraction in the cell is denoted as α_q , then the following three conditions are possible:

- $\alpha_q = 0$: the cell is empty (of the q th fluid).
- $\alpha_q = 1$: the cell is full (of the q th fluid)
- $0 < \alpha_q < 1$: the cell contains the interface between the q th fluid and one or more other fluids.

Based on the local value of α_q , the appropriate properties and variables are assigned to each control volume within the domain.

4.3.2. The Volume Fraction Equation

The tracking of the interface(s) between the phases is accomplished by the solution of a continuity equation for the volume fraction of one (or more) of the phases. For the q th phase, this equation has the following form:

$$\frac{\partial \alpha_q}{\partial t} + \vec{v} \cdot \nabla \alpha_q = 0 \quad (4.1)$$

The volume fraction equation is not solved for the primary phase; the primary-phase volume fraction is computed based on the following constraint:

$$\sum_{q=1}^n \alpha_q = 1 \quad (4.2)$$

4.3.3. Properties of Fluids

The physical properties of each fluid are calculated as weighted averages based on the volume fraction of the individual fluid in a single cell. The fluid volume in a cell is computed as $F_{vol} = F V_{cell}$, where V_{cell} is the volume of a computational cell and F is the liquid volume fraction in a cell. The value of F in a cell should range between 1 and 0 and $F = 1$ represents a cell which is completely filled with water and $F = 0$ represents a cell which is completely filled with oil and $0 < F < 1$ represents the interface between oil and water.

The liquid volume fraction is determined by solving a separate passive transport equation, given as:

$$\frac{\partial F}{\partial t} + \vec{F} \cdot \nabla F = 0 \quad (4.3)$$

where,

$$F = \frac{\text{cell volume occupied by water}}{\text{total volume of the control cell}} \quad (4.4)$$

The mixture's physical properties are derived from that of the two phases through the volume fraction function. In particular, the average value of ρ and μ in a computational cell can be computed from the value of F in accordance with:

$$\rho = F \rho_2 + (1 - F) \rho_1 \quad (4.5)$$

$$\mu = F \mu_2 + (1 - F) \mu_1 \quad (4.6)$$

where the subscripts 1 and 2 represent the water and oil phases, respectively.

4.3.4. The Momentum Equation

A single momentum equation is solved throughout the domain, and the resulting velocity field is shared among the phases. The momentum equation, shown below, is dependent on the volume fractions of all phases through the properties ρ and μ . The flow is considered to be laminar, incompressible, Newtonian and isothermal with velocity field $\vec{v}$ governed by the Navier-Stokes and continuity equations, which can be written as:

$$\frac{\partial \rho \vec{v}}{\partial t} + \nabla \cdot (\rho \vec{v} \vec{v}) = -\nabla P + \rho g + \nabla \cdot (\mu (\nabla \vec{v} + \nabla^T \vec{v})) + \vec{F}_s \quad (4.7)$$

where $\vec{v}$ is the velocity of the mixture, P the pressure, t the time, $\vec{F}_s$ the volumetric force at the interface resulting from surface tension, ρ the density and μ dynamic viscosity. In Eq. 4.7, the accumulation and convective momentum terms in every control volume (cell) balance the pressure force, gravity force, shear force, and additional surface tension force $\vec{F}_s$.

4.3.5. Surface Tension and Wall Adhesion

The VOF model can also include the effects of surface tension along the interface between each pair of phases. The model can be augmented by the additional specification of the contact angles between the phases and the walls.

Surface Tension

Surface tension arises as a result of attractive forces between molecules in a fluid. The surface tension model in FLUENT is the continuum surface force (CSF) model proposed by Brackbill et al.[79]. With this model, the addition of surface tension to the VOF calculation results in a source term in the momentum equation. To understand the origin of the source term, consider the special case where the surface tension is constant along the surface, and where only the forces normal to the interface are considered. It can be shown that the pressure drop across the surface depends upon the surface tension coefficient, σ , and the surface curvature as measured by two radii in orthogonal directions, R_1 and R_2 :

$$p_2 - p_1 = \sigma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (4.8)$$

where p_1 and p_2 are the pressures in the two fluids on either side of the interface.

In FLUENT, a formulation of the CSF model is used, where the surface curvature is computed from local gradients in the surface normal at the interface. Let n be the surface normal, defined as the gradient of α_q , the volume fraction of the q th phase.

$$n = \nabla \alpha_q \quad (4.9)$$

The curvature, κ , is defined in terms of the divergence of the unit normal, $\hat{n}$ ([79]):

$$\kappa = \nabla \cdot \hat{n} \quad (4.10)$$

where

$$\hat{n} = \frac{n}{|n|} \quad (4.11)$$

The surface tension can be written in terms of the pressure jump across the surface. The force at the surface can be expressed as a volume force using the divergence theorem. It is this volume force that is the source term which is added to the momentum equation.

Wall Adhesion

An option to specify a wall adhesion angle in conjunction with the surface tension model is also available in the VOF model. The model is taken from work done by Brackbill et al.[79]. Rather than impose this boundary condition at the wall itself, the contact angle that the fluid is assumed to make with the wall is used to adjust the surface normal in cells near the wall. This so-called dynamic boundary condition results in the adjustment of the curvature of the surface near the wall.

If θ_w is the contact angle at the wall, then the surface normal at the live cell next to the wall is

$$\hat{n} = \hat{n}_w \cos \theta_w + \hat{t}_w \sin \theta_w \quad (4.12)$$

where $\hat{n}_w$ and $\hat{t}_w$ are the unit vectors normal and tangential to the wall, respectively. The combination of this contact angle with the normally calculated surface normal one cell away from the wall determine the local curvature of the surface, and this curvature is used

to adjust the body force term in the surface tension calculation.

Discretization

The governing equations are discretized to algebraic equations by using a control-volume-based technique. The PISO (Pressure-Implicit with Splitting of Operators) algorithm is used in the transient calculations. The technique of Geo-Reconstruct scheme (Piecewise-Linear Interface Construction (PLIC)) is used for the surface tension calculations adopted in this model for the accuracy of the oil-water interface.

Time Dependence

Each time step value Δt used in the simulation was 10^{-4} and the Courant number condition was kept at 2. The Courant number is a dimensionless number that compares the time step in a calculation to the characteristic time of transit of a fluid element across a control volume:

$$\frac{\Delta t}{\Delta x_{\text{cell}}/v_{\text{fluid}}} \quad (4.13)$$

In the region near the fluid interface, FLUENT divides the volume of each cell by the sum of the outgoing fluxes. The resulting time represents the time it would take for the fluid to empty out of the cell. The smallest such time is used as the characteristic time of transit for a fluid element across a control volume, as described above. Based upon this time and input for the maximum allowed Courant Number, a time step is computed for use in the VOF calculation.

Boundary Conditions

No-slip wall boundary conditions were used for the simulations. Mass flow rate of the continuous phase (oil) and the dispersed phase were specified at the inlets of the microchannel according to the capillary number ($Ca = \mu U / \sigma$, where Ca is the capillary number of the flow, μ is the viscosity of the continuous phase, and U velocity of the continuous phase and σ is the interfacial tension between the dispersed phase and the continuous phase) of the flow. Flow rate of deionized water (dispersed phase) was kept constant at 50 $\mu\text{l/hr}$ during the experiments and simulations and the capillary number was varied by changing the flow rate of the oil (continuous phase). The outlet boundary condition of the channel is set to normal

atmospheric pressure.

The following fluids and their properties were used for both simulations and experiments : 1) Mineral oil as the carrier fluid (M5904, Sigma-Aldrich) with 2% w/w Span 80 surfactant (Sigma-Aldrich S6760) and 2) DI water with fluorescent dye (0.05% w/w Acid Yellow). Hydrodynamic properties: Viscosity of DI water (μ) is 1 mPa s, interfacial tension between water and mineral oil is 3.65 mNm⁻¹, contact angle between water and PDMS is 115° as the PDMS used in the experiment is hydrophobic and without any surface treatment, viscosity of mineral oil with 2% w/w Span 80 is 23.8 mPa.s. These fluid properties were set in the fluent properties panel for the fluent VOF simulations. The VOF fluent simulations were run from Ca=0.008 to 0.025. Mesh independence study was conducted to ensure the result obtained are independent of the mesh size.

4.3.6. Mesh Independence

Mesh independence study was conducted to ensure the results obtained are independent of the mesh size. Totally five mesh sizes were considered. They were (i) 12.5 μm ; (ii) 10 μm ; (iii) 6.7 μm ; (iv) 5 μm and (v) 4 μm . The mesh size was for both the height and width directions and the mesh size in the length of the channel direction was kept constant at 10 μm to reduce the computational requirements, since the flow rate was low and the channel length was high. Pressure in the side channel, P_d , was chosen for the mesh independence study because the main focus of this investigation is to explore the nature of the pressure profile during the droplet formation process. Figure 4.2 shows the pressure measured in the side channel as a function of time. It can be seen that the pressure measured for 12.5 μm mesh size shows large variations in the pressure and as the mesh size decreases to 10 μm the fluctuations reduce and for the mesh size of 6.7 μm , 5 μm and 4 μm , it exhibits very little variation. Therefore, taking into consideration the computational requirement, the mesh size of 6.7 μm was chosen for all the simulations done during this investigation.

4.4. Experimental details

4.4.1. Fabrication

The channel designs were printed into a photolithographic mask and the negative SU-8 photo resist (Microchem Corp.) was used to fabricate the master mold using standard procedures

Figure 4.2. *Grid independence test conducted for P_d with mesh size varying from 12.5 μ m to 4 μ m.*

specified from Microchem. Then microfluidic chips were fabricated using polydimethylsiloxane (PDMS) polymer (Dow corning Sylgard 184 Silicone Elastomer) through the standard soft lithography process for PDMS microchannel fabrication explained in chapter 3. The cured PDMS microchannels were bonded to another piece of flat PDMS layer after treating them with oxygen plasma. And they are allowed to recover their hydrophobicity, because of the need of the walls to be hydrophobic which facilitates the formation of water droplets in oil.

4.4.2. Experimental setup and testing

The continuous phase (oil) and the dispersed phase (deionized water) were pumped from gas-tight syringes (Hamilton, 1.25ml) through the tubing (0.8 mm PTFE, Cole-Parmer) connected to the inlets, with the help of syringe pumps (KD Scientific, Model No. 781200). Capillary number of the flow was changed by changing the volume flow rate of the continuous phase. The DI water with fluorescent stream forms droplets at the T-junction. The experiments were observed under the Inverted Fluorescence Microscope (Nikon Eclipse TE2000-S) with suitable magnification using Plan Apro objectives. The visualisation of the experiments were captured and recorded through the eye piece of the inverted microscope using a CCD camera (DCRDVD803E, SONY) camera and used for the analysis of the droplet break-up process.

4.5. Validation of numerical simulation with experimental flow visualisation

Simulation results are compared with the experimental results obtained for the same geometry of the microchannel. Figure 4.3 shows the experimental flow visualisation on the left and the simulation results on the right for $Ca = 0.01$. As can be seen in the figure, the flow visualisation of the simulated results for droplet formation match very well with the flow visualisation of the experimental results. The time for the experimental visualisation figure (a) is taken to be the same as the time of the simulation figure (b) and the further calculations were done using the time elapsed between the images in the experimental video.

The process of formation of droplets in the T-junction, as seen in Fig. 4.3, can be described as follows. The two immiscible fluids (oil and water) form an interface at the junction of the inlet and the main channels. The stream of the dispersed phase (water)

penetrates into the main channel and a droplet begins to grow; the pressure gradient and the flow in the main channel distort the droplet in the downstream direction. The interface on the upstream side of the droplet moves downstream. When the interface approaches the downstream edge of the inlet for the dispersed phase, the neck connecting the inlet channel with the droplet breaks. The disconnected liquid plug flows downstream in the main channel, while the tip of the stream of the dispersed phase retracts to the end of the inlet and the process repeats. The experimental visualization and the numerical simulation and the process of droplet break-up confirms to the results observed by Garstecki et al.[9].

4.6. Pressure profiles in the dispersed and continuous fluids during droplet break-up

Now that we have validated the simulated results with the experimental results and the droplet break-up process confirms to the established results, we can use the pressures measured in the dispersed phase (p_d) and in the continuous phase (p_c) to understand the process of the droplet break-up and the underlying physics. The pressures p_d , p_c were measured at positions seen in Fig. 4.1(b). The pressures measured at these positions were recorded over several seconds and the plotted values are a sample of the measured values.

4.7. Squeezing regime

Figure 4.3 shows the 2D experimental (right) and VOF (right) flow visualizations during the droplet formation process from $t = 24.3 \text{ ms}$ to $t = 27.5 \text{ ms}$ for $Ca=0.01$. All of the VOF flow visualisations were taken at the mid plane of the microchannel (i.e. $50 \text{ }\mu\text{m}$ from both walls). Figure 4.4 shows the evolution of pressures in the continuous phase (p_c) and the dispersed phase (p_d) and the difference between (p_d-p_c) them for $Ca=0.01$, which falls under the squeezing regime. These two figures are to be referred together to see how p_c , p_d and (p_d-p_c) change during the droplet break-up process. We can start from $t = 24.3 \text{ ms}$ when the droplet is about to break as seen in Fig. 4.3(a,b). This is the final moment of the droplet break-up after squeezing has taken place.

After the droplet break-up, the dispersed phase recoils into the inlet of the side channel and as it recovers the pressure for the next cycle, its pressure p_d jumps up to a higher level;

but pressure p_c comes down because the droplet, after the break-up, moves down with the flow and provides less resistance to the flow of the continuous phase. Therefore, the difference between p_d and p_c reaches the maximum before the start of the next cycle as seen at point (I) in Fig. 4.4. Now, during the start of the droplet formation process, the dispersed phase starts to push itself into the main channel and the pressure p_d slowly comes down. But the continuous phase pressure p_c starts climbing slowly to adjust to the incoming dispersed phase which increasingly occupies more area as the droplet formation process proceeds. The sudden fall in pressure for both p_d and p_c , between $t=25.4$ ms and $t=26.3$ ms, is due to a droplet, which had been formed before, leaving the channel at the outlet. This is because the pressure drop across the channel is dependent on the number of droplets in the channel ([80]). Once the droplet leaves the outlet both p_d and p_c regain their pressure. But the difference between, p_d-p_c , slowly decreases as the droplet emerges from the side channel into the main channel.

In the blocking phase (stage II) — where the dispersed phase starts blocking (Fig. 4.3(g) and point (II) in Fig. 4.4) the main channel significantly and spans the whole cross-section of the main channel ($\varepsilon < w$) — p_d gradually decreases as the dispersed is pushed into the main channel and p_c increases to the point. In this stage p_d-p_c declines linearly with a steep slope and the process proceeds to the squeezing stage (Fig. 4.3(i),(j); stage (III) in Fig. 4.4). In the squeezing stage as the droplet elongates in the downstream direction and the neck connecting it to the inlet thins p_d-p_c remains almost constant, and paving the way for the continuous pushing of the dispersed phase till the final stage of break-up happens (Fig. 4.3(m),(n); and stage (IV) in Fig. 4.4). At this point both p_d and p_c come down and their difference p_d-p_c is at the lowest value, unlike suggested by other researchers that it happens between the second and third stage of the droplet formation process ([9]).

From the above observation four stages of droplet formation can be identified in terms of the pressure difference p_d-p_c as seen in Fig. 4.4: entering stage (I), where p_d-p_c decreases slowly; blocking stage (II), where p_d-p_c decreases steeply; elongation stage (III), where p_d-p_c remains almost constant; droplet break-up stage (IV), where p_d-p_c reaches the lowest value.

After the break-up, the dispersed phase recoils back to the inlet and the above described process repeats. Fluctuations in p_d and p_c are clearly seen throughout the droplet formation process in Fig. 4.4. The reason for fluctuations in p_d and p_c can be explained as follows: As the droplets tracked in the flow is comparable to the size of the channel, they block and provide resistance to the flow of the dispersed phase. As these droplets are moving

Figure 4.3. Comparison of flow visualization obtained from experiment (left) and simulation (right) showing the four stages of formation of droplet in the squeezing regime ($Ca=0.01$): (I) the stream of dispersed phase enters into the main channel (a,b), (II) the stream blocks the main channel (g,h), (III) the droplet elongates and grows downstream (k,l), (IV) the droplet break-up (m,n)

Figure 4.4. *Evolution of pressures in the continuous phase p_c and the dispersed phase p_d and the difference between them ($p_d - p_c$) for $Ca=0.01$, and the four stages of formation of droplet in the squeezing regime: (I) the stream of dispersed phase enters into the main channel, (II) the stream blocks the main channel, (III) the droplet elongates and grows downstream, (IV) the droplet break-up*

and are coupled to the flow in momentum and continuity equation, the competition between the two phases leads to fluctuations in both p_d and p_c . In incompressible fluids pressure disturbances are transmitted instantly and therefore, fluctuations in $p_d - p_c$ is less compared to the individual pressure fluctuations.

4.8. Transition regime

Figures 4.5 and 4.6 show the pressures p_d and p_c measured and the difference $p_d - p_c$ for $Ca=0.014$ and $Ca=0.018$, respectively. Figure 4.7 shows the experimental (left) and numerical (right) flow visualizations for $Ca=0.018$. It can be seen that the experimental visualizations match to the VOF simulated visualizations. In the transient regime, the dynamics of droplet break-up is dominated by both the shear force and the squeezing mechanism. The dispersed fluid from the side channel blocks the main channel partially and the size of the droplet produced is significantly smaller than in the squeezing regime. The time required for droplet formation is also reduced as the capillary number is increased. It can be observed from figures 4.4, 4.5 and 4.6 that, as the capillary number increases, the length of the pressure curve for the dispersed phase p_d and the time required for recoiling and regaining the pressure for the next cycle slowly decreases. This is because the velocity of the fluid and the shear stress it exerts on the incoming fluid from the dispersed phase increases with increase in capillary number; the squeezing phenomenon gradually starts disappearing — not an abrupt change, but gradual — until the droplet formation process moves to the dripping regime. This happens along with the increase in the pressures of p_d and p_c . The individual pressures of p_d and p_c at higher capillary numbers may be high but the pressure difference between them remains almost constant as seen in Fig. 4.11. In the transition regime, the stages of droplet formation cannot be clearly distinguished in terms of the pressure difference $p_d - p_c$, as in the squeezing regime, because of almost a linear decline as seen in Fig. 4.5 and Fig. 4.6. The lowest difference for $p_d - p_c$ happens at the moment of droplet break-up.

4.9. Dripping regime

In the dripping regime, where the role of shear stresses becomes more important, the contribution by the pressure build up to droplet break-up becomes lower but nevertheless exist and never becomes negligible, unless the radius of the droplet is much smaller than the width

Figure 4.5. *Evolution of pressures in the continuous phase p_c and the dispersed phase p_d and the difference between them ($p_d - p_c$) for $Ca=0.014$*

Figure 4.6. *Evolution of pressures in the continuous phase p_c and the dispersed phase p_d and the difference between them ($p_d - p_c$) for $Ca=0.018$*

Figure 4.7. *Snapshots of flow visualizations in the transition regime ($Ca=0.018$): experimental (left) and the VOF simulation (right)*

Figure 4.8. *Evolution of pressures in the continuous phase p_c and the dispersed phase p_d and the difference between them ($p_d - p_c$) for $Ca=0.022$*

Figure 4.9. *Evolution of pressures in the continuous phase p_c and the dispersed phase p_d and the difference between them ($p_d - p_c$) for $Ca=0.025$*

Figure 4.10. Snapshots of flow visualizations in the dripping regime ($Ca=0.025$): experimental (left) and the VOF simulation (right)

of the channel ([50]). But in the confined channel the droplet size is comparable to the size of the microchannel and the droplet break-up occurs due to both shear stress and pressure build-up. Fig. 4.10 shows both the experimental (left) and the VOF flow visualisations (right) for $Ca=0.025$, where the process of droplet formation and the break-up with respect to time can be referred together with Fig. 4.9. The droplet clearly does not occupy the main channel fully as in the squeezing regime as seen in Fig. 4.10 and the volumetric rate of flow of the continuous phase makes it to flow faster in gap between the droplet and the wall of the channel and exerts a larger shear stress on the droplet. And the size of droplet in this regime is comparatively lower than in the squeezing and the transition regime.

The fluctuations in the pressure of both dispersed and continuous phase in the dripping regime decrease as the capillary number increases. This result is consistent with the observation made by De Menech et al. [50]. During the droplet formation process, after the pressure recovery, p_d continues to decrease and p_c continues increase till they reach the point where the droplet breaks up. The sudden drop in both p_d and p_c is due to a droplet leaving the channel exit as explained in the squeezing regime. In the dripping regime, the pressure difference p_d-p_c decreases in a linear fashion and the stages of droplet formation cannot be identified, as is the case for this regime.

4.10. Effect of Ca on p_d-p_c

Figure 4.11 shows the difference between the pressures measured in the dispersed phase and continuous phase p_d-p_c for capillary numbers between 0.01 and 0.025. As seen in figure 4.11, the difference in the pressures are bounded within a range and doesn't vary much with the increase in capillary number. The time for forming a droplet decreases as the capillary number increases. This result is similar to the results obtained by De Menech et al.[50]. They observed that for a fixed viscosity ratio as the capillary number increases parameters such as squeezing time remains unchanged. Therefore, the characteristics that the pressure difference remains for a wide range of capillary number could be attributed to the viscosity ratio, i.e. for a given viscosity ratio the pressure difference required to form droplets in a wide range of capillary numbers remains constant.

Figure 4.11. *Effect of Ca on the difference of dispersed phase and continuous phase pressures ($p_d - p_c$)*

4.11. Summary

Our numerical investigation of the dynamics of droplet break-up in the T-junction of a microchannel has revealed that the evolution of pressure in the dispersed phase varies as much as the continuous phase pressure in the squeezing regime, transition regime and in the dripping regime. The evolution of pressure over time in the continuous phase and the flow visualization confirms to the model proposed by other researchers. Therefore, the pressure measured in the dispersed phase represents the true picture of the droplet break-up process in a microfluidic T-junction. From the variation of the dispersed phase pressure we could see that the assumption of constant dispersed phase pressure leads to an unrealistic picture, though it allowed to explain the droplet formation process in simple terms. The difference between the dispersed phase pressure and the continuous phase pressure shows us that the droplet formation process in a T-junction starts with a higher pressure difference and as the process proceeds to form a droplet the difference reduces gradually as the droplet formation proceeds. The lowest difference between the p_d and p_c happens at the moment of droplet break-up, and not between the second and third stage of the droplet formation process as suggested by other researchers. The findings of this study are important for multiphase microfluidics, as the understanding of droplet formation and the physics behind the process are essential for designing integrated multiphase microfluidic systems.

5. Merging Reagents into Droplets - Experimental Results

This work is published in the journal of Microfluidics and Nanofluidics ([81]).

5.1. Introduction

Merging reagents into the droplets moving in the mainchannel at a T-junction (Fig. 4.2(a)) experience problems like synchronization of droplet arrival, contamination of the injecting stream and reliable merging only in a narrow range of flow rates ([14, 42, 60]). Replacing single T-junction with multi-junction eliminates the need for synchronisation ([13]), but has higher fabrication cost due to the insertion of hydrophilic side channels separately. Injecting reagents alternatively from two side branches of double T-junctions increases the synchronization frequency in a wide range of flow rates but merging is not guaranteed to 100% at all flow rate conditions ([61]). In this chapter the experimental results obtained for merging reagents into droplets at a T-junction with expansion right after the junction is presented. This design exploits the basic fluid flow physics in the microchannel to increase the reliability of adding reagents into droplets.

5.1.1. Microchannel Design

The rational behind the provision of an expanded section lies in the answer to the following question: Why do extra droplets form? If the merging has to happen on a continuous basis at the T-junction, droplet formation from the side channel has to be synchronized ([42]) with the arrival of the droplet which has already been formed and moving in the mainchannel. Otherwise an extra droplet forms, as seen in Fig. 5.1(a), termed as unreliable merging, a state of merging where the reagent itself forms a droplet. Garstecki et al.[49] proposed that the breakup of the two immiscible liquids at the T-junction in droplet microfluidics is dominated

by the pressure built up across the droplet as it forms at low values of the capillary number ($< 10^{-2}$). Similarly, merging reagents into droplets with conventional T-junction, which has similar dynamics as that of the droplet formation at a T-junction, pressure builds up across the emerging reagent droplet due to the high resistance to the flow of continuous fluid in the thin films that separate the droplet from the walls of the microchannel, when the droplet fills almost the entire cross-section of the channel. This pressure buildup squeezes the droplet to break from the T-junction when the already formed droplet approaches it, as seen in Fig. 5.9(c).

Therefore, if the pressure build-up that squeezes the droplet to detach it from the T-junction can be reduced, we can avoid the extra droplet problem. We provide an expanded section, just after the T-junction, on both sides of the channel: the expansion in the side which is opposite to the droplet forming side channel provides the extra space for the carrier fluid to move forward, as seen in Fig. 5.1(b), so that the pressure build-up can be reduced; the expansion in the side from which the droplet forming provide the space for the droplet to grow, which allows the space for the extra volume created during the waiting time for the droplet arrival in the main channel. In doing that, there is one more additional feature added, apart from the reduced pressure drop: when the droplet breaks before merging, due to longer waiting time and droplets growing big to block the extra space, the expanded section can function like a time delay component. It can restrict the movement of the droplets and facilitate the process of droplet coalescence. Fig. 5.1 shows the schematic diagram of the microchannel designs (a) with conventional T-junction and (b) with an expanded section. There are three inlets to pump the fluids into the microchannel and one outlet to collect the spent fluids. The mineral oil inlet and the DI water with fluorescence inlet meet at the Y-junction and the DI water inlet from the side meet the main channel at T-junction. The expanded section is located at the distance of $50\mu\text{m}$ after the T-junction, but could be located as closely as possible, the constraint being the resolution of the photo mask. The microchannel has the height of $100\mu\text{m}$. The other dimensions of the microchannel are shown in Fig. 5.1

All dimensions are in μm

Figure 5.1. *Schematic of the droplet merging channel: a) conventional T-junction b) T-junction with expansion. ΔP_L is the Laplace pressure between the oil and DI water interface*

5.2. Experimental details

5.2.1. Fabrication

The channel designs were printed into a photolithographic mask and the negative SU-8 photo resist (Microchem Corp.) was used to fabricate the master mold using standard procedures specified by Microchem. Then microfluidic chips were fabricated using polydimethylsiloxane (PDMS) polymer (Dow corning Sylgard 184 Silicone Elastomer) through the standard soft lithography process for PDMS microchannel fabrication as described in section 3.2. The cured PDMS microchannels were bonded to another piece of flat PDMS layer after treating them with oxygen plasma. And they were allowed to recover their hydrophobicity, because of the need of the walls to be hydrophobic which facilitates the formation of water droplets in oil.

5.2.2. Experimental setup

The following fluids were used for the experiments: 1) Mineral oil as the carrier fluid (M5904, Sigma-Aldrich) with 2% w/w Span 80 surfactant (Sigma-Aldrich S6760), 2) DI water with fluorescent dye (0.05% w/w Acid Yellow) and 3) DI water. Hydrodynamic properties: Viscosity of DI water (μ) is 1 mPa s, interfacial tension between water and mineral oil is 3.65 mNm⁻¹, contact angle between water and PDMS is 115°, viscosity of mineral oil with 2% w/w Span 80 is 23.8 mPa.s.

The fluids were pumped from gas-tight syringes (Hamilton, 1.25ml) through the tubing (0.8 mm PTFE, Cole-Parmer) connected to inlets, with the help of syringe pumps (KD Scientific, Model No. 781200). The DI water with fluorescent stream forms droplets (droplet A) at the Y-junction as seen in Fig. 5.1. When the fluorescent droplets formed at the Y-junction reach the T-junction, the aqueous reagent stream (DI water) from the side channel is merged to the droplets. The experiments were observed under the Inverted Fluorescence Microscope (Nikon Eclipse TE2000-S) with suitable magnification using Plan Apro objectives and mercury lamp for illumination and blue filter for visualisation. The visualisation of the experiments were captured and recorded through the eye piece of the inverted microscope using a CCD camera (DCRDVD803E, SONY) and used for the analysis of the reliability of droplet merging process.

5.3. Testing

Experiments were carried out for the T-junction with expanded section and for the conventional T-junction designs. The combination of fluorescent stream flow rates (Q_{fluo}) and DI water flow rates (Q_{DI}) were from 0 $\mu\text{l/hr}$ to 250 $\mu\text{l/hr}$ in the interval of 25 $\mu\text{l/hr}$, and the mineral oil flow rate was maintained at the ratio of 1.5 to the fluorescent flow rate. Between 0 $\mu\text{l/hr}$ to 25 $\mu\text{l/hr}$, intermediate flow rates of 10 $\mu\text{l/hr}$ to 20 $\mu\text{l/hr}$ were also used to measure the merging percentage. The flow rate ratio (FR) between mineral oil and fluorescent was kept constant at 1.5 with the aim that the droplets should be not too long or too short for this merging process. We found this ratio by changing the ratio from 0.1 to 4.0, with keeping the flow rate of fluorescence constant at 50 $\mu\text{l/hr}$. The corresponding picture is shown in Fig. 5.2, in which it is seen that the flow rate ratio from 1 to 2.5 produce droplet which are not too long or too short. We chose 1.5 and kept constant throughout the experiments. The videos of the experiments were recorded for both the conventional T-junction and T-junction with expanded section designs.

Figure 5.2. *Droplet length vs flow rate ratios of mineral oil and fluorescence*

5.4. Results and discussion

5.4.1. Reliability of the merging process

The recorded videos of the experiments were used to calculate the reliability of the merging process by counting the number of successfully merged droplets. The percentage of reliable merging for a particular flow rate combination was calculated as follows,

$$\%Reliability = \left(\frac{\text{number of successfully merged droplets}}{\text{total number of droplets generated for merging}} \right) \times 100. \quad (5.1)$$

The contour lines indicating percent rate of droplet merging for T-junction alone and T-junction followed by an expansion of 150 μm were constructed and are shown in Figs. 5.3 and 5.4. Reliable merging (100%) for T-junction happens in a very narrow range of flow rates (below the dashed contour line) as seen in Fig. 5.3 and merging in a wide range of flow rate ratio gives the extra droplet problems. Microchannel with T-junction followed by an expansion provides reliable merging in a wide range of flow rates and flow rate ratio as seen in Fig. 5.4. The region of reliable merging is wide (region between the contour line of value 98%) and droplets are merged reliably in a wide range of fluorescent flow rates except at high DI water flow rates (bottom right) and low fluorescent flow rates (top-left). It is because at higher flow rates of DI water, not enough fluorescent droplets (droplet A) are formed. The low fluorescent stream cannot overcome the pressure drop of carrier fluid (mineral oil) as well the pressure drop due to high DI water flow rate to form droplets at the Y-junction. Therefore the merging percentage is low for high DI water flow rate at the top-left region in Fig. 5.4. But when the flow rate becomes slightly higher, fluorescent stream can overcome the pressure drop and form enough droplets to merge reliably.

Outside the high percentage success, in general, there are three scenarios: 1) when the DI water flow rate is high and the fluorescent flow rate is low, extra droplets (droplet B) form and the merging percentage goes down; when the DI water flow rate is very high, only droplet B forms, which is not desirable as no merging is possible. 2) when the fluorescent flow rate changes from very high compared to DI water flow rate, DI water stream cannot overcome the pressure drop to merge continuously with droplet A. 3) when both fluorescent flow rate and DI water flow rate are very high, stratified flow occurs, which is not desirable

Figure 5.3. *Contour plot for merging of reagents into droplets with conventional T-junction*

for the merging process.

5.4.2. Droplet volumes and droplet formation time

Fig. 5.5 shows a sample of measurements for the volume of the droplets generated at the Y-junction for various flow rates of DI water with mineral oil flow rate fixed at the ratio of 1.5 with respect to the fluorescence flow rate for the droplet merging process. The droplet generated are consistent over time with volumes in the order of few nanolitres with less than 10% standard deviation as seen in Fig. 5.5. Fig. 5.6 shows the droplet volumes generated for various flow rates of fluorescence from the side channel at the T-junction with expansion and without expansion. These droplet volumes in the range of few nanolitres were measured from the experiments without the merging process. The volume of the droplets generated at the T-junction with expansion is higher than the volume of droplets generated at T-junction without expansion.

Fig. 5.7 shows the droplet formation time at the T-junction with expansion and without expansion. As expected, the droplet formation time at the T-junction with expansion is higher than the time for the T-junction without expansion. This increase in volume and time is due

Figure 5.4. *Contour plot for merging with T-junction and subsequent expansion*

Figure 5.5. *Volume of the droplets formed at the Y-junction*

to the expanded section, which reduces the pressure drop and the availability of extra space for droplet growth leads to longer residence time for the droplet at the T-junction.

Figure 5.6. *Critical volume of the droplets formed at the conventional T-junction and T-junction with expansion*

The reliability of the merging process with an expanded section was analysed by measuring the droplet length before merging and after merging. Fig. 5.8 shows the droplet lengths measured before merging and after merging for different fluorescent flow rates and DI water flow rates. As we can see from Fig. 5.8, the length of droplets (droplet A) generated at the Y-junction (before merging) is consistent and the droplet volume decreases with the increase in the fluorescence flow rate. This also reflects in the amount of DI water merged into it at the T-junction with expansion. As the droplet length goes down, the amount of DI water merged to it also goes down because of the small residence time for the droplet at the T-junction. As the DI water flow rate is increased, the amount of DI water added to the same length of droplet also goes up due to higher volume flow rate of DI water. The length of droplets after the merging process is also consistent as seen in Fig. 5.8.

5.4.3. Flow physics in the merging process

Figure 5.9(a-c) shows the sequence of droplet break-up due to pressure build up at a conventional T-junction. Figure 5.9(a) shows a droplet (A) approaching the droplet which is forming from the side channel (B). Due to blockage of space by droplet B and the need for the carrier fluid to flow the pressure in the channel builds up to squeeze the carrier fluid

Figure 5.7. *Time for droplet formation at the conventional T-junction and T-junction with expansion*

Figure 5.8. *Droplet lengths before merging and after merging for various flow rates of fluorescence and DI water*

between droplet B and the channel wall. This high pressure also acts on droplet B forming from the side channel and when the force due to pressure is beyond the surface tension force which is holding the droplet at the T-junction, the droplet breaks away as seen in figure

5.9(b) and (c). This happens when the volume flow rate in the sidechannel is high and the consequent asynchronous arrival of the droplet A. Therefore, the conventional T-junction merges the droplets reliably in a narrow range of flow rates of both continuous and dispersed phases.

The increase in the reliability of the merging process in the T-junction with expanded section design can be explained as follows. The expanded region right after the T-junction provides an extra space between the droplet forming from the side channel and the wall and allows the carrier fluid to move forward avoiding the pressure build up beyond threshold-level and break-up of droplet B from the T-junction. Now, two things can happen for the droplet forming from the side channel:

1. droplet sticks to the T-junction due to surface tension and grows in the expanded region (Fig. 5.9(d-e)) until the droplet A merges with it (Fig. 5.9(f)).
2. droplet breaks away because of high droplet volume (Fig. 5.10(a)), due to longer waiting time for the droplet A to arrive.

In the first case, after merging, the merged droplets are squeezed from the T-junction due to pressure build up across the droplet as in the normal droplet formation process, because now the merged droplet fully occupies the channel, and flows normally through the expanded section and to the outlet of the channel.

In the second case, droplet B and droplet A coalesce in the expanded region. The expanded region facilitates the droplets to come closer and coalesce, as seen in (Fig. 5.10(b)), because the normal channel after the expansion acts like a time delay component and delays the forward movement of the extra-droplet in the microchannel. This is basically a passive flow trapping method and the expanded section helps the droplets come closer together by acting as reservoir for the continuous phase between the droplets to drain away. When the droplets contact each other, their contacting surface gets flattened and when they separate a low pressure region is created and the droplets fusion occurs. The process of droplet fusion in an expanded rectangular section was reported and explained in detail by Bremond et al. [58]. When the delay time of droplet A is long, extra droplets of B occur and the merging percentage goes down. The sequence of droplet coalescence is seen in Fig. 5.10(a-d).

The expanded section not only eliminates the extra droplet formation but also enhances droplet merging and can be explained as follows: the extra space provided by the expanded region reduces the pressure build up that happens in the droplet formation at the T-junction

Figure 5.9. *Droplet merging phenomena in: (a-c) - extra droplet formation in a conventional T-junction, (d-f) - merging of droplets before break off in a T-junction followed by an expanded section*

Figure 5.10. *Coalescence of droplets in the expanded section after droplet break-up*

and it is less than the threshold value for droplet break-up; the carrier fluid movement through the extra space exerts shear stress on the droplet forming from the side channel; therefore,

the droplet formation dynamics has been changed by the expanded section at the T-junction from purely pressure dominant break-up to a combination of pressure drop and shear due to the flow of carrier fluid; the combination of shear stress and pressure buildup distorts the interface ([49]) and makes it flat, as seen in Fig. 5.9(d). The distortion remains until the fluorescent droplet (droplet A) hits the emerging DI water droplet (droplet B).

When the interface is flat, the curvature becomes low and the radius of curvature approaches infinity; based on the Young-Laplace equation, Laplace pressure jump ΔP_L exerted by the interface on the emerging droplet in a rectangular channel can be described as, $\Delta P_L = \sigma (1/R_w + 1/R_h)$, where σ is the interfacial tension between the two phases, R_w and R_h are interface curvatures in width and height directions respectively; Laplace pressure jump, ΔP_L , across the interface is negligibly small and when the fluorescent droplet (droplet A) hits the DI water droplet (droplet B) at the T-junction with expansion, they merge easily without breaking the DI water droplet to form extra droplet. Small Laplace pressure jump across the interface lowers the disturbance needed to rupture the thin film between the droplets and it reduces the possibility of droplet B breaking from the T-junction at the moment droplet A hits it. When the external pressure increases above the internal pressure at a point on the droplet surface, the droplets break up and the constituents merge.

5.4.4. Enhanced mixing

The merging and the coalescence patterns of droplets due to expanded section enhances mixing, because after merging the merged fluids are in axial arrangement inside the droplets as seen in Figs. 5.9(f) and 5.10(d). Tanthapanichakoon et al.[82] reported that axial arrangement of droplet constituents enhances mixing and it can be attributed to the reduction of the striation length between reactant segment by interlayering. Similar results for enhanced mixing due to axial arrangement (in-line droplet fusion) have been reported by Liu and Frenz et al.[56, 83]. Therefore, it can be stated that axial arrangement of droplet constituents in this merging design lead to enhanced mixing.

5.5. Summary

In summary, a T-junction design with expansion has been demonstrated that exploits the physics of fluid flow phenomena in microchannels to eliminate the drawbacks in adding

reagents into the droplets at a T-junction. An expanded section, right after the T-junction, reduces the pressure build up and increases the residence time of the droplet at the T-junction; in the case of droplet break-up, it facilitates the coalescence of the droplets and reduces the probability of the formation of extra droplet. Holding the droplet at the T-junction leads to the distortion of the interface of the emerging droplet and becomes flat because of the shear stress and pressure build-up due to the moving carrier fluid. A flat interface, according to the Laplace-Young equation, results in low Laplace pressure between two phases and enhances the merging of miscible droplets. Reagent addition in this design leads to axial arrangement of the droplets constituents in the merging as well as in the coalescence process and facilitates faster mixing of reactants. Therefore, the demonstrated design provides a better alternative for the available merging schemes and can be effectively used in the microfluidic chips used for biological, bio-chemical and μ -TAS assays.

6. Merging Reagents into Droplets - Computational Results

6.1. Introduction

Merging reagents into the droplets moving in the mainchannel at a T-junction (Fig. 6.1(a)) experience problems like synchronization of droplet arrival, contamination of the injecting stream and reliable merging only in a narrow range of flow rates ([14, 42, 60]). To overcome the problems, Sivasamy et al.[81] reported, as presented in chapter 5, a microchannel design that exploits the basic fluid flow physics in the microchannel to increase the reliability of adding reagents to droplets. They demonstrated that an expansion in the microchannel right after the T-junction enhances the reliability in adding reagents to the preformed droplets at a T-junction. In this chapter, the reported phenomena is numerically investigated and compared with the experimental results to validate the computational results. The physics of the fluid flow and merging reagents to droplets at a T-junction is explained through the computationally observed pressure measurements and flow visualization.

6.2. Geometry of the microchannel

Figure 6.1(a) and 6.1(b) show the schematic of the microchannels with conventional T-junction and T-junction with expansion. The microchannel dimensions of the main channel are $100\text{ }\mu\text{m}$ in height, $200\text{ }\mu\text{m}$ in width and the length used is $1000\text{ }\mu\text{m}$ and the side channel is $100\text{ }\mu\text{m}$ in width and since the microchannel is planar the height is the same as of the main channel (figures not drawn to scale). The dimensions of the channel were chosen keeping in mind the conditions provided by Garstecki et al. [9] that the width of the main channel should be greater than its height and the width of the inlet channel should be at least equal to half the width of the main channel.

Figure 6.1. *2D schematic illustrations of the three dimensional microchannel used in both experiment and computation: a) conventional T-junction; b) T-junction with an expanded section*

Merging reagents from a side channel to droplets (A) at a conventional T-junction in microchannels, as shown in Fig. 6.1(a) and Fig. 6.2, face problems like extra droplet formation – the reagent stream from the side channel itself forming droplets (B) – and reliable addition of reagents happening only in a narrow range of flow rates of reagents and the droplet forming fluid. Providing an expanded section right after the T-junction alleviates the above described problems and reliably adds reagents to droplets over a wide range of flow rates of both liquids ([81]). The rationale for providing the expanded section to address the problem of extra droplets (B) can be explained as follows: extra droplets form when the incoming reagent stream from the side channel of the T-junction breaks to form a droplet because of the pressure build-up in continuous phase due to blocking of the channel. Fig. 6.2(b) shows the velocity flow field at the T-junction when the droplet forming from the side channel is about to break after going through stages of droplet formation process at a T-junction in the squeezing regime ([9]). As the gap between the incoming reagent droplet (B) and channel wall becomes significantly low, the continuous phase flow faces a higher resistance moving forward and the velocity vectors are projected towards the incoming dispersed fluid, as seen in Fig. 6.2(a,b), and creates the pressure buildup for the droplet to break, seen in Fig.

Figure 6.2. *Experimental (left) and VOF simulation (right) of extra droplet formation at a conventional T-junction. VOF simulation shows the velocity vectors, responsible for creating the pressure buildup for the droplet breakup, projecting at the incoming dispersed phase (a); and formed extra droplet (b)*

6.2(c,d), leading to extra droplet problem. But, when an extra space is provided through an expansion right after the junction, the side that is opposite to the side channel provides the space for the continuous phase to move forward without creating pressure build-up and the expansion in the same side as that of the side channel provides the space for the extra volume of droplet that is growing, as seen in Fig. 6.1(b), extra droplet problem can be eliminated and successful merging of reagents to droplets can be achieved in a wide range of flow rates.

6.3. Experimental details

6.3.1. Fabrication

The channel designs were printed into a photolithographic mask and the negative SU-8 photo resist (Microchem Corp.) was used to fabricate the master mold using standard procedures specified from Microchem. Then microfluidic chips were fabricated using polydimethylsiloxane (PDMS) polymer (Dow corning Sylgard 184 Silicone Elastomer) through the standard soft lithography process for PDMS microchannel fabrication as explained in section 3.1.1. The cured PDMS microchannels were bonded to another piece of flat PDMS layer after treating

them with oxygen plasma. And they are allowed to recover their hydrophobicity, because of the need of the walls to be hydrophobic which facilitates the formation of water droplets in oil.

6.3.2. Experimental setup and testing

The oil and the deionized water were pumped from gas-tight syringes (Hamilton, 1 ml) through the tubing (0.5 mm PTFE, Cole-Parmer) connected to inlets, with the help of syringe pumps (KD Scientific, Model No. 781200). The continuous phase (oil) is pumped through the main channel and the dispersed phase (DI water with fluorescence) is pumped through the side channel and forms droplets and flow in the main channel. The fluorescent droplets (droplet A, see Fig. 6.1(b)) approach the T-junction where reagent stream (DI water) is pumped with the help of syringe pumps. Since both droplet A and the reagent stream are of the same fluid — they are miscible and the addition of fluorescence to water does not change its properties — droplet A merge with the reagent at the T-junction. The experiments were observed under the Inverted Fluorescence Microscope (Nikon Eclipse TE2000-S) with suitable magnification using Plan Apro objectives and mercury lamp for illumination and blue filter for visualisation. The visualisation of the experiments were captured and recorded through the eye piece of the inverted microscope using a CCD camera (DCRDVD803E, SONY) and used for the analysis of the reagent addition process to droplets.

6.4. Numerical simulation

6.4.1. Governing equations

The transient three-dimensional numerical simulations of the multiphase flow of three fluids (mineral oil and DI water with fluorescence and pure DI water) in microchannel with T-junction are performed using VOF method available in the commercial software FLUENT (Ansys Inc. USA). The VOF model can model two or more immiscible fluids by solving a single set of momentum equations and tracking the volume fraction of each of the fluids throughout the domain. Interaction between oil and water phases is considered as non-interpenetrating and since DI water with fluorescence and pure DI water are miscible, they are considered as single phase for the convenience of computation. Since the experiments are conducted in micro scale dimensions VOF model with surface tension effects is incorporated

for the computations. The governing equations, fluid properties, incorporation of surface tension and wall adhesion, boundary conditions were similar to the description in section 4.3

The following fluids and their properties were used for both simulations and experiments : 1) Mineral oil as the carrier fluid (M5904, Sigma-Aldrich) with 2% w/w Span 80 surfactant (Sigma-Aldrich S6760), 2) DI water with fluorescent dye (0.05% w/w Acid Yellow) and 3) pure DI water. Hydrodynamic properties: Viscosity of DI water (μ) is 1 mPa s, interfacial tension between water and mineral oil is 3.65mNm^{-1} , contact angle between water and PDMS is 115° as the PDMS used in the experiment is hydrophobic and without any surface treatment, viscosity of mineral oil with 2% w/w Span 80 is 23.8 mPa.s. These fluid properties were set in the fluent properties panel for the fluent VOF simulations. The VOF fluent simulations were run for fluorescent stream flow rates (Q_{flu}) and DI water flow rates (Q_{DI}) from $0\text{ }\mu\text{l/hr}$ to $250\text{ }\mu\text{l/hr}$ in the interval of $25\text{ }\mu\text{l/hr}$, and the mineral oil flow rate was maintained at the ratio of 1.5 to the fluorescent flow rate ([81]). Each time step value Δt used in the simulation was 10^{-4} and the Courant number condition ($\text{CFL} = \Delta t * u / \Delta x$, where CFL is the Courant number; Δt is the time step value; u is the velocity of the fluid flow; Δx is the mesh size) was kept at 2.

6.5. Reagents addition to droplets at the T-junction with an expanded section

6.5.1. Merging reagents to droplets at the T-junction with an expanded section

Figures 6.2 and 6.3 show the experimental (left) and the numerical (right) flow visualizations of merging reagents (B) into substrate droplets (A) at a conventional T-junction and at a T-junction with expansion in a microchannel, respectively. As we can see from the figures, the numerical flow visualizations match with the experimental flow visualizations. Merging reagents with the substrate droplet at a conventional T-junction happens in the following sequence: the substrate droplet approaches the T-junction where the reagent stream is being pushed into the main channel; and if the substrate droplet's arrival is synchronized with the reagent droplet's formation at the T-junction they merge with each other resulting in a successful reagent addition. But if the substrate droplet's arrival is not synchronised – higher flow rates of reagent stream – then extra droplet forms, resulting in an unreliable reagent addition operation as seen in Fig. 6.2. But if an expanded section is provided, the reagent

droplet formation time is increased due to the availability of extra space for the reagent stream to flow without facing the pressure buildup; volume of the reagent droplet to remain at the T-junction without breaking; leading to a successful operation in a wide range of flow rates for both droplet forming liquid (Q_{fluo}) and reagent liquid (Q_{DI}) as seen in Figs.6.3(e,f;g,h).

6.5.2. Droplet coalescence in the expanded section

Fig. 6.4 shows the experimental (left) and the numerical (right) flow visualizations of coalescence of reagent droplet (B) and substrate droplet (A) at the T-junction with expansion in a microchannel. The numerical flow visualizations match with the experimental flow visualizations of the droplet coalescence in the expanded region of the microchannel. At the event of longer waiting time for the substrate droplet (A) to arrive, due to higher flow rate of the reagent stream and the lower flow rate of the substrate droplet forming fluid, the reagent droplet (B) breaks up, as seen in Fig. 6.4(e,f); and they coalesce in the expanded region as seen in Fig. 6.4(g,h). The other figures show that after the coalescence process the flow in the microchannel would continue as in the normal operating conditions. Because, the expansion after the T-junction acts a time delay component and facilitates droplet coalescence in the expanded region and improving the reliability of the reagent addition operation to a wider range of flow rates than a conventional T-junction would do ([81]).

6.6. Physics of droplet merging

The flow physics of merging reagents to microfluidic droplets at a T-junction with expansion right after the junction has been intuitively explained by Sivasamy et al.[81]. To demonstrate the flow physics that is responsible for the reliable operation of reagent merging a systematic numerical study was conducted. Here we show that the flow field near the T-junction at the time of reagent droplet formation creates the favourable conditions responsible for reliably adding reagents to droplets in a wide range of operating conditions. A 2D schematic illustration of droplet formation with an expanded section in the three dimensional microchannel taken for this study is shown in Fig. 6.5. Pressure measurements were made in the continuous phase, the dispersed phase and inside the droplet. The notations p_d in the side channel, p_c and p_{dr} in the main channel are for the positions at which the dispersed phase pressure, the continuous phase pressure and the pressure in the droplet are measured and they are located

Figure 6.3. *Snapshots of flow visualizations of reagent addition to droplets at a T-junction: experimental (left) and the VOF simulation (right)*

Figure 6.4. *Snapshots of flow visualizations of droplet coalescence in the expanded section: experimental (left) and the VOF simulation (right)*

Figure 6.5. *An illustration of the two dimensional T-junction used in computation*

at 50 μm from both upper and lower walls (i.e. mid-plane of the three-dimensional channel).

Fig. 6.6 shows the experimental and computational visualizations of droplet formation at the T-junction with expansion for $\text{Ca}=0.01$ ($\text{Ca}=\mu U/\sigma$, where Ca is the capillary number of the flow, μ is the viscosity of the continuous phase, and U velocity of the continuous phase and σ is the interfacial tension between the dispersed phase and the continuous phase) and they both match qualitatively well. Fig. 6.7 shows the pressure measurements made for p_d , p_c and p_{dr} at the positions seen in Fig. 6.5. We intentionally left out droplet A for this analysis because of the need to eliminate the confusion that might arise in the understanding of the pressure measurements when the droplet merges with the reagent stream (droplet B). The process of enhanced droplet merging can be explained, with the help of Figs. 6.7, 6.8 and 6.9 as follows: the extra space provided by the expanded region reduces the pressure build up that happens in the droplet formation at the conventional T-junction and it is less than the threshold value for droplet break-up; the carrier fluid movement through the extra space and the velocity vectors parallel to the incoming reagent fluid surface exert shear stress on the droplet forming from the side channel, as seen in Fig. 6.9(a); therefore, the droplet (droplet B) formation dynamics has been changed by the expanded section at the T-junction from purely pressure dominant break-up to a combination of pressure drop and shear due to the flow of carrier fluid; the combination of shear stress and pressure buildup distorts the interface [49] and makes it flat, as seen in Fig. 6.6(i,j;k,l).

When the interface is flat, the curvature becomes low and the radius of curvature approaches infinity; based on the Young-Laplace equation, Laplace pressure jump ΔP_L exerted by the interface on the emerging droplet in a rectangular channel can be described as, $\Delta P_L = \sigma (1/R_w + 1/R_h)$, where σ is the interfacial tension between the two phases, R_w

Figure 6.6. *Experimental (left) and VOF flow visualizations (right) of the droplet formation process at a T-junction with an expanded section for $Ca=0.01$*

Figure 6.7. Pressures measured for the droplet formation at a T-junction with expansion and at the positions seen in Fig. 6.5

Figure 6.8. *Pressure measurements in the zoom in version of the region showed in Fig. 6.7*

Figure 6.9. *Velocity flow field around a droplet forming at the T-junction expansion in a microchannel: (a) velocity vectors responsible for creating the pressure buildup for the droplet breakup are moving away from the incoming dispersed phase and thus creates a scenario where the droplet breakup is a combination of both shear stress and pressure build up, which is significantly less compared to the conventional T-junction, and not purely based on pressure buildup-based squeezing mechanism; (b) successful merging of droplet and the reagent stream without extra droplet*

and R_h are interface curvatures in width and height directions respectively; Laplace pressure jump, ΔP_L , across the interface is negligibly small. This can be observed in Fig. 6.7, in the zoom in region, and in Fig. 6.8, where the pressure measured inside the droplet (P_{dr}) is approaching the pressure measured in the continuous phase (P_c) when the droplet is about to break. This can be identified by observing $P_d - P_c$, which remains almost constant for a longer duration — the time when droplet B is growing in to the expanded region — and reaches the lowest value at the moment of droplet breakup [78]; and also the time when P_d starts rising for the next cycle of droplet formation. The sudden dip in P_{dr} below the value of P_c happens after the droplet break-up, as seen in Fig. 6.8, because when the droplet breaks away from the T-junction, there is a sudden acceleration of the continuous phase which causes a negative pressure relative to the operating pressure in the region before the continuous phase fills up the region. When the substrate droplet (droplet A) hits the DI water droplet (droplet B) at the T-junction with expansion, they merge easily without breaking the DI water droplet to form extra droplet. Because, small Laplace pressure jump across the interface lowers the disturbance needed to rupture the thin film between the droplets and it reduces the possibility of droplet B breaking from the T-junction at the moment droplet A hits it. When the external pressure increases above the internal pressure at a point on the droplet surface, the droplets break up and the constituents merge successfully as seen in Fig. 6.9(b).

6.7. Summary

In this chapter, simulated results of the reagent addition process to droplets at a conventional microchannel T-junction and a T-junction with expansion right after it is reported. It has been observed that the numerically simulated results using the VOF model match with the experimental flow visualizations. Experimentally observed problems such as extra droplets formation and the physics behind the problem in the conventional T-junction have been simulated numerically. The reliable operation using an expanded section at the T-junction reported in the earlier research ([81]) has been verified numerically. The flow field and the pressure measurements have been used to understand the physics of fluid flow responsible for reliable operation. The above findings from the numerical investigation would help in designing and operation of reagent addition to droplets in multiphase microfluidic systems.

7. A simple method for evaluating and predicting chaotic advection in microfluidic slugs

This work is published in the journal of Chemical Engineering Science ([84]).

7.1. Introduction

Many applications for lab-on-a-chip require liquids of more than one phase which are not related chemically or misible with each other. Droplet and gas-liquid segmented flow are the two kinds of flow that have been investigated for passive micromixing of liquids as they are known to mix the components rapidly by internal recirculation ([20, 68, 85, 49, 45, 86]). Laminar flow conditions, which is prevalent in continuous microchannel flows, can be observed in droplet micromixing ([39]). Segmented gas-liquid flow, which is often called slug flow according to the flow conditions, also exhibits laminar flow conditions. In droplet flows, the liquid inside the droplet generates two counter rotating vortices. In the case of gas-liquid segmented flow, the liquid slug between two gas bubbles generates the counter rotating vortices in the Taylor flow regime ([67]).

Many of the studies in microfluidics literature are application-driven experimental studies involving complex geometries with little or no theory ([87]). And it is well known that many theoretical and computational studies found in basic fluid mechanics and physics literature tend to focus on basic phenomena in simple and well characterized geometries. Very few researchers have tried to model the chaotic motion in slugs and droplets moving in microfluidic channels. Experimental scaling of the chaotic mixing in a droplet moving through a winding microfluidic channel has been done using baker's transformation ([20]). The scaling argument for the dependence of mixing time is, $t \sim (aw/v) \log(Pe)$, where w is the cross-

setional dimension of the microchannel, a is the dimensionless length of the slug measured relative to w , v , is the flow velocity, Pe is the Péclet number ($Pe = wv/D$) and D_{mol} is the diffusion coefficient of the reagent being mixed. However, the argument was too simple and does not provide the optimization of the channel geometry for rapid mixing through chaotic advection. A mathematical model to estimate the mixing time for slugs in a straight slit microchannel was proposed ([23]). However, the model did not provide any insight about chaotic motion in the slugs. The mixing characteristics inside a microfluidic slug using computational fluid dynamics was done by Tanthapanichakoon et al.[82], where each slug was modelled as a single-phase flow domain in two-dimensional (2D) as well as in three-dimensional (3D) domain. Boundary conditions of the slug in Lagrangian frame of reference were used to simulate the mixing characteristics. They reported that the radially arranged reactants mix more rapidly than the axially arranged reactants and proposed a new dimensionless number to estimate mixing rates. However, they did not calculate chaotic advection of the flow inside the slugs and also the dimensionless number proposed does not provide insight to predict a channel geometry for rapid mixing. Since droplets and slugs produce similar vortices, similar inferences can be made from their flowfields when moving in various microchannel geometries and hence only the slug terminology will be used for convenience hereafter.

In order to understand mixing in microfluidic slugs, we propose a simple method, based on the geometry of the channel, to find whether a particular geometry produces chaotic advection or not. The case of a slug moving in a rectangular channel having high aspect ratio (β) (slit microchannel) is taken and the velocity flow field far from the boundary wall of the longest dimension is assumed as two dimensional (2D). Though the internal recirculating flow field inside the slug is three-dimensional ([65]), the 2D assumption holds good in the case of slit microchannel. Analytical solution for the two-dimensional flow field is derived and the boundary conditions mimicking the flow of the slug in microchannel is used to get the velocity at every point of the flow domain. The 2D analytical solution is compared and validated against a 2D slice of the 3D slug flow field far from the boundary walls, using the commercial CFD code, Fluent 6.3.26 (ANSYS, Inc., USA). Velocity field obtained from the analytical model is used to track the passive tracer particles and construct Poincaré maps and dye advection pattern. To analyse the nature of chaotic advection, traditional tools such as ‘Variance’, ‘Shannon Entropy’ and ‘Complete Spatial Randomness’ are used.

7.2. Problem Description

Microchannel flows are similar to Stokes flow problems because of the inherently laminar flow conditions. Gas-liquid segmented flow (often called as slug flow) can be described by Stokes equations, when the liquid-slug is considered as a single phase flow in Lagrangian frame of reference, which model viscous fluids in macroscales and ordinary fluids in microscales. We consider the case where a slug fully occupies the channel cross-section and its cross sectional shape is determined by that of the channel. The microchannel is considered as infinitely large in one of the three directions and therefore the flow in a plane far from the boundary walls, in the direction, is two-dimensional. Fig. 7.1 shows the model of a liquid slug with moving wall boundary conditions in Lagrangian frame of reference in a straight rectangular microchannel. The continuous phase is assumed to wet the wall well and no-slip condition is applied on the top and the bottom of the slug. In the front and the rear, the slug is surrounded by an immiscible phase with negligible viscosity such as air and therefore no shear stress condition is assumed. Other assumptions made in this analysis are: no body force has any influence on the slug; the slug is approximately rectangular; the liquid in the slug is an incompressible Newtonian fluid. Recirculation happens in the form of two vortices in both sides of the slug, because the slug makes contact with the wall of the microchannel with no-slip condition and in the lagrangian frame of reference one can observe the vortices clearly. Here, we consider a slug moving in a slit microchannel ($(w \gg h)$) and flow field in a plane far from the boundary walls is modelled as two-dimensional low-Reynolds-number flow (Stokes flow).

7.3. Modelling

7.3.1. 2D analytical solution

Following the works of Shankar[88] and Timoshenko[89], the governing equation for Stokes flow in the slug can be simplified as a biharmonic equation. Using the Finite Fourier transform (FFT) method, Sivasamy et al. and Che et al.[84, 24] found the dimensionless velocities in the x and z directions as follows (see Appendix A) :

1. Moving top wall 2. Moving bottom wall 3. Moving side wall (outer) 4. Moving side wall (inner)

a) High aspect ratio channel with $w \gg h$ b) Vortices in the x - z plane far from the side boundary walls

Figure 7.1. *3D model of the slug in a slit microchannel simplified into 2D model with internal recirculating flow with appropriate boundary conditions on moving walls and no shear stress conditions on front and rear edge. The slug is moving in positive x -direction and the wall velocities are in negative x -direction*

$$\begin{aligned} \hat{u}_x = \frac{\partial}{\partial z} \hat{\varphi}(\hat{x}, \hat{z}) = \sum_{n=1}^{\infty} \sin(\alpha_n \hat{x}) \\ \times [C_{1n} \alpha_n \sinh(\alpha_n \hat{z}) + C_{2n} \alpha_n \cosh(\alpha_n \hat{z}) + C_{3n} \alpha_n \hat{z} \sinh(\alpha_n \hat{z}) \\ + C_{3n} \cosh(\alpha_n \hat{z}) + C_{4n} \alpha_n \hat{z} \cosh(\alpha_n \hat{z}) + C_{4n} \sinh(\alpha_n \hat{z})] \end{aligned} \quad (7.1)$$

$$\begin{aligned} \hat{u}_z = -\frac{\partial}{\partial x} \hat{\varphi}(\hat{x}, \hat{z}) = -\sum_{n=1}^{\infty} \cos(\alpha_n \hat{x}) \times \\ (C_{1n} \cosh(\alpha_n \hat{z}) + C_{2n} \sinh(\alpha_n \hat{z}) \\ + C_{3n} \hat{z} \cosh(\alpha_n \hat{z}) + C_{4n} \hat{z} \sinh(\alpha_n \hat{z})) \end{aligned} \quad (7.2)$$

The constant coefficients can be determined from the boundary conditions Eq. A.8 and Eq. A.10,

$$C_{1n} = 0 \quad (7.3)$$

$$C_{2n} = -\frac{4\xi}{D_n\beta\alpha_n} \sinh(\alpha_n) + \frac{4\eta}{D_n\beta} [\sinh^2(\alpha_n) - \cosh^2(\alpha_n)] \quad (7.4)$$

$$C_{3n} = \frac{4\xi}{D_n\beta} \sinh(\alpha_n) + \frac{4\eta \sinh^2(\alpha_n)}{\beta\alpha_n D_n} \quad (7.5)$$

$$C_{4n} = \frac{4\xi}{D_n\beta\alpha_n} [-\alpha_n \cosh(\alpha_n) + \sinh(\alpha_n)] \\ + \frac{4\xi}{D_n\beta\alpha_n} [-\sinh(\alpha_n) \cosh(\alpha_n) - \alpha_n \sinh^2(\alpha_n) + \alpha_n \cosh^2(\alpha_n)] \quad (7.6)$$

where,

$$D_n = \alpha_n^2 \cosh^2(\alpha_n) - (\alpha_n^2 + 1) \sinh^2(\alpha_n) \quad (7.7)$$

7.3.2. 3D Numerical simulation

To verify the validity of the model described above, the analytical flow field is compared with the numerically simulated flow field using the commercial CFD software Fluent 6.3.26 (ANSYS, Inc., USA). The dimensions of the slug taken for the simulation are $100 \times 200 \times 50 \mu\text{m}$ in x , y and z directions respectively. Therefore, l:w:h of the slug is 2:4:1 and in $x - z$ direction, the 2D plane in consideration is of the ratio 2:1. The front and rear end of the slug are assumed to be straight edges, as the effect of the edge curvatures is small ([82]). Steady-state simulation condition was assumed. The number of meshes used for the simulation was 64000. The mesh independence was confirmed. The density and viscosity were set at 998.2 kg/m^3 and 0.001 Pa.s respectively as water is taken as the operating fluid. Since the Reynolds number is very low in the microfluidic slugs, the laminar flow model was used. The moving wall boundary conditions for all the four walls were set at 5 mm/s and for the front and the rear ends, no-shear wall conditions were applied. Convergence criteria for x , y and z velocity values were set at 10^{-5} . Slices of the three-dimensional numerical simulation of the flow field inside the slug is shown in Fig. 7.2. The contour slices are showing the flow field in the $x - z$ plane from wall to wall in the Y axis.

Figure 7.2. 2D slices of 3D flow field in the slug model at different Y planes

7.3.3. Comparison

For comparing the analytical flow field with the numerically computed flow field, we take the flow field at the middle plane, which is far from the boundary walls. Therefore, numerical velocity flow field from the plane in the y -axis at $y = 0.0001$ m ($100 \mu\text{m}$) was taken for comparison.

Flow field for both analytical and numerical solution are shown in figures 7.3(a) and 7.3(b) respectively. For the analytical velocity flow field, the moving wall velocity boundary conditions on the top (U_{top}) and the bottom (U_{bottom}) of the slug are both 5 mm/s and the aspect ratio of the slug $\beta = 2$, which are same as used for numerical simulation. As seen in figures 7.3(a) and 7.3(b), both analytical and numerical flow field contours and the velocity vectors are similar. To make sure that they are of the same magnitude, velocity profiles at three different positions along x axis are compared. Velocity profile plots of analytical and numerical flow fields at $1/4$, $1/2$ and $3/4$ positions of the slug are shown in Fig. 7.4. The continuous outer x axis in Fig. 7.4 stands for the position on the slug where the velocity profile is drawn and the three discrete inner x axes stand for the magnitude of the velocity in that particular position of the slug. As seen in Fig. 7.4, both analytical and numerical velocity profiles are of the same magnitude with little variation in the centre. Therefore, the 2-D simplification of the flow field for the slug flow in slit microchannel is valid and can be

used to analyse the chaotic advection by tracking passive tracer particles in the slug.

Figure 7.3. *Velocity field inside the slug: (a) analytical result (b) numerical result at the middle plane ($y=0.0001$ m)*

7.4. Particle tracking

In this study, we use passive massless tracers to construct the particle trajectories in the flow domain using Lagrangian particle tracking method. The velocity expression used for integration is,

Figure 7.4. *Comparison between analytical and numerical results of the velocity profile at different x positions (Velocity values in the inner x -axis and position of the velocity profile in the outer x -axis).*

$$\dot{x} = v(x, t) \quad (7.8)$$

where x is the position vector, v is the velocity, t is the time, and the dot denotes a material derivative. Here, we use only the analytically known Eulerian velocity field, therefore the integration can be carried out by the standard fourth order Runge-Kutta method. Boundary conditions used for finding the analytical velocity field, which is used to track the particles in the slugs, are discussed in the next section. The constant integration time, $\Delta t = 0.02$ s, was found by running a few trials of computation for the range of boundary wall velocities used in this study. The choice of integration time Δt is dictated by: 1) the accuracy of tracing the particle along the streamline in a non-chaotic flow field without moving out of it, i.e., the particle has to simply trace the streamline pattern, 2) the highest among the values, to avoid the need for huge computational resources.

7.4.1. Boundary conditions

Analytical velocity flow fields, for tracking passive tracer particles, were found by using the boundary conditions which mimic the kind of motion the slug undergoes in microchannels. For straight microchannels, the slug experiences a constant velocity on the walls in Lagrangian

frame of reference. Therefore a constant velocity of -5 mm/s was used in Eq. 7.2, along with the constant coefficients for finding the velocity flow field. Though the slug is moving in the positive x-direction, the velocity boundary condition is negative because the frame of reference is moving with the slug. For meandering microchannel, in the first half period, the outer wall moves at a higher speed (V_{R_1}) and inner wall moves at a lower speed (V_{R_2}). In the second half, the wall velocities reverse. Effect of change in the wall velocity reflects in the size of the vortices formed in a slug as shown in Fig. 7.5(a). Boundary conditions mimicking the motion of a slug through a meandering channel and straight channel for a single period are shown in Fig. 7.5(b). The difference between the wall velocities is dependent on the dimensions of the microchannel such as radius, width and the angular velocity of the slug. The outer radius, inner radius and width of the channel is 1000 μm , 800 μm and 200 μm respectively. Angular velocity of the slug is $\omega=1$ rad/s. The inner and outer wall velocities were calculated from the relation, $V_S = (V_{R_1} + V_{R_2})/2$, where V_S is the straight channel wall velocity.

7.5. Chaotic advection evaluation

7.5.1. Poincaré map

Poincaré map, a Lagrangian tool constructed by recording the particle positions for long time, is used to evaluate the advective transport of fluid particles. The disposition of the particle positions in Poincaré map reveals the nature of the flow: chaotic nature of the flow field appears as randomly distributed; non-chaotic flow appears as islands or closed curves ([63, 64]). Since we use massless tracer particles, when the flow is steady, the particle trajectories correspond to stream lines and hence no chaotic advection. Fig. 7.6(a) is the Poincaré map for straight channel velocity boundary condition, which shows that particles move in closed curves.

When the flow is unsteady and chaotic, stretching and folding of fluid elements occurs and produces an exponential growth of the fluid interface and the particle positions are randomly distributed. In this study, Poincaré sections are obtained by following the motion of 20 material points, which are put in a group at the location (1.4,0.4) in a slug for $\beta = 2$, for the duration of 2000 periods. An impression of the poincaré sections that have been found for straight microchannel and meandering microchannel can be seen in figures 7.6(a)

- 1) Small inner vortex and big outer vortex
- 2) Equal size vortices
- 3) Small outer vortex and big inner vortex

(a)

(b)

Figure 7.5. (a) Schematic of vortices in a slug moving in a meandering microchannel (b) Boundary conditions for the slug moving in a straight channel and meandering channel

Figure 7.6. *Poincaré map for (a) slug flow in straight microchannel (b) slug flow in meandering channel*

and 7.6(b) respectively.

7.5.2. Construction of dye advection pattern maps

Advection pattern of massless passive tracers are obtained by tracking the positions of 40,000 material points which are initially concentrated in a rectangular box of size 0.05×0.05 centered at the point $(1.45, 0.45)$. Blue and red colour particles are used to distinguish the upper and lower half particles in the rectangular box similar to the work by Kang et al.[90] and it is useful to visually identify the globally chaotic flow. In this study, 40 periods of meandering channel boundary conditions with 630 iterations per period are used to track the particles in the slug. Particle positions were recorded at the end of each period of tracking to see the

particle advection patterns. Figures 7.7(a-j) show the dye advection patterns for the slug of aspect ratio $\beta=2$ for periods from 1 to 40 with interval of 8 periods.

7.6. Results and Discussion

As seen in Fig. 7.6(a), Poincaré map constructed for straight channel shows that the particles in the slug move along with streamlines. This is because of the velocity flow field for constant wall velocity boundary on the slug that produces two vortices with symmetrical recirculation along the channel centerline. In a simple non-chaotic recirculating flow field, particles can move from one half to the other can happen only through diffusion. And the particles used for particle tracking in this study are passive massless tracers with zero diffusivity and therefore they simply follow the flow field.

Poincaré map for meandering channel flow is seen in Fig. 7.6(b) and the particles are evenly distributed throughout the domain. Using the traditional approach of visually analyzing Poincaré sections shows that there are no islands and can be classified as globally chaotic flow. Total number of particles in the Poincaré map is 40,000 which exceeds the requirement of sufficient number particle positions, $n > M \ln(M) = 5348$, for evaluating Poincaré maps([76]), where the number of bins $M=800$. Fig. 7.6 shows the 'Variance Index' and 'Shannon Entropy Index' for the dye advection patterns, as seen in Fig. 7.7, computed for the slug from velocity flow field obtained for the meandering channel flow. Both mixing measures show that the mixing is complete and approach the state of 'Complete Spatial Randomness'. Therefore, it is clear that the meandering channel geometry is useful in chaotic mixing due to its ability to create assymetrical vortices.

7.7. Optimization

In section 7.6, it has been established, through modelling of fluid flow inside a slug, that the meandering channel can indeed be used for promoting chaotic advection. But there are parameters associated with slug micromixing which can affect the chaotic advection. For example, slugs and plugs can be generated in a wide range of aspect ratios and the velocity of the slugs moving the channel can also vary according to the operating conditions. Therefore, an attempt to find out the optimal parameters is made in this section. The aspect ratio of the slug and the velocity of the slug are the two parameters taken for optimization as these

Figure 7.7. (a-j) Dye advection pattern for the slug aspect ratio $\beta=2$ for 40 periods: (a)= 0, b=1, c=5, d=10, e=15, f=20, g=25, h=30, i=35, j=40.

Figure 7.8. *Variance index I_{var} and b) Shannon entropy index of periods N for the dye advection pattern in Fig. 7.7*

are the two characteristics of slugs that can be changed easily in microchannels.

Aspect ratios of the slug taken for optimization are from $\beta=1, 2, 4, 6, 8$ and 10 . Aspect ratios of slug less than 1 tend to produce spherical droplets and they experience less internal circulation as they do not make sufficient contact with the channel walls. Therefore, aspect ratios of slug more than 1 is considered in this investigation. The velocity of slug ω , for one cycle of the meandering channel, is considered from the value of $\pi/6, \pi/5, \pi/4, \pi/3, \pi/2$ and π radians/sec. Optimization is done by keeping one variable constant and changing the other, meaning when the aspect ratio β is held constant. For example, the velocity of the slug ω is changed, through the boundary conditions applied for particle tracking, to see how does it affect the chaotic advection inside the slug, and vice versa.

7.7.1. Effect of slug velocity

Figures 7.9, 7.10 show the calculated variance index and Shannon entropy index for aspect ratios from 1 to 10 and the velocity of the slugs from π to $\pi/6$. As can be seen in Fig. 7.9, for aspect ratios from $\beta=1$ to 10 , variance index decreases as mixing takes place in the slug as it moves through the meandering channel. For the aspect ratio of $\beta=1$, the variance index decreases rapidly and reach the $I_{var,CSR}$ limit quickly and the corresponding Shannon entropy index also reaches the $I_{S,CSR}$ limit in the same manner for $\omega=\pi/2$ as seen in 7.9(a) and 7.10(a) respectively. For $\beta=1$ and the velocity $\omega=\pi$, mixing occurs at a slower rate than for $\omega=\pi/2$. For the same aspect ratio as the slug velocity is decreased, the rate of mixing

Figure 7.9. (a-f) Comparison of the effect of velocity on chaotic advection for slug aspect ratio between 1 and 10 through variance index

gets slower and does seem to approach the $I_{var,CSR}$ and $I_{S,CSR}$ limits asymptotically.

As β increases from 1 to 2 and 4, as seen in figures 7.9(a), 7.9(b), 7.9(c), the rate of mixing gradually decreases for higher velocities but attains a level for $\beta=4$ for which the rate of mixing is independent of the velocity of the slug. It can also be observed that the assumption taken for the experiments in section 5.3, that the slugs need to be not too long or too short for reagent addition and mixing is indeed valid. This is clearly seen in Fig. 4.9 where the measured slug lengths before and after reagent addition range from $700\mu\text{m}$ to $900\mu\text{m}$. For a channel width of $200\mu\text{m}$ used in the experiments, the aspect ratio falls in the range of approximately 4, for which, of course, the rate of mixing is independent of the velocity of the slug or the capillary number of the flow. Therefore, the result obtained in this optimization process can indeed be applied in the real experimental conditions. Because, once we add reagents to slugs they are expected to be mixed quickly and if the rate of mixing is independent of the velocity or flow rate of the continuous phase and capillary number, then the uncertainty in the mixing time can be eliminated, where there is a need to use a wide range of flow rates of the continuous phase and the dispersed phase.

As the aspect ratio of the slug, β is increased to 6, 8 and 10, the rate of mixing gradually decreases because as the aspect ratio increases the time taken for the particles to travel the whole volume of the slug increases and the consequence of that is the long mixing time. And the change in velocity of the slug has a very little effect and mixing takes as much as 40 cycles of the microchannel for the variance index to reach the $I_{var,CSR}$ limit — the limit for complete mixing. Therefore, it is clear that the velocity of the slug does have an effect on chaotic advection and the rate of mixing becomes almost independent of the velocity for $\beta=4$.

7.7.2. Effect of Aspect Ratio

Figures 7.11 and 7.12 show the effect of aspect ratio on chaotic advection for slug aspect ratio from 1 to 10 through variance index and the Shannon entropy index respectively. As seen in figures 7.11(a) and 7.11(b) the variance index for aspect ratio $\beta=1$ decreases rapidly and reaches the $I_{var,CSR}$ limit faster than other aspect ratios for $\omega = \pi$ and $\omega = \pi/2$. This trend can also be seen in Shannon entropy index reaching the $I_{S,CSR}$ limit in figures 7.12(a) and 7.12(b). However, when the velocity decreases to $\omega = \pi/3$ and less, slug aspect ratios of 2 and 4 take the lead and reach the $I_{var,CSR}$ limit faster than other aspect ratios as seen in

Figure 7.10. (a-f) Comparison of the effect of velocity on chaotic advection for slug aspect ratio between 1 and 10 through entropy index

figures 7.11(c), 7.11(d), 7.11(e) and 7.11(f). The same trend can be seen in figures 7.12(c), 7.12(d), 7.12(e) and 7.12(f) where Shannon entropy indices reach the $I_{S,CSR}$ limit quickly for $\beta=2$ and 4. From these observations, it is clear that the aspect ratio of the slug does have a profound effect on the chaotic advection inside the slug.

For a low aspect ratio of $\beta=1$, higher velocities tend to advect the massless passive particles effectively inside the slug and lead to better mixing in a less number of meandering channel cycles. In other words, lower aspect ratio slugs need higher velocities to transfer wall shear to the liquid inside it and hence higher internal recirculation. But, at intermediate aspect ratios of 2 and 4, the chaotic advection of the particles becomes independent of velocity of the slug as seen in section 7.7.1. Though for the aspect ratio of 2 it is not clearly evident in figures 7.9(b) and 7.10(b), but in figures 7.11(c), 7.11(d), 7.11(e) and 7.11(f) it is evident that for all the velocities below $\pi/3$, variance index for $\beta=2$ and 4 reaches the $I_{var,CSR}$ limit in less number of cycles than other aspect ratios. Therefore, it is clear that the intermediate slug aspect ratios of 2 and 4 tend to promote chaotic advection efficiently than the smaller aspect ratios and bigger aspect ratios. However, better mixing can be achieved in smaller aspect ratio slugs if the velocity of the slug is kept higher. If the velocity needs to be slow, then intermediate slug aspect ratios are preferred.

7.8. Predicting a channel geometry for chaotic mixing

Researchers have investigated various three dimensional microchannel designs for their efficacy to increase mixing rate. For example, Xia et al.[91] have investigated different three dimensional channels and found that the mixers can be chaotic, partially chaotic or non-chaotic. Therefore, the out of the plane meandering or bending of channel not necessarily increase the mixing rate. The microchannel designs have to be investigated independently for their effect on increased mixing rate.

Therefore, we investigate channel designs by applying the two-dimensional model in the section 7.3 along with the boundary conditions for a particular channel geometry to identify the channel geometry which enhances chaotic mixing in the slug, by tracking particles in the velocity flow field as described in this work. Boundary conditions mimicking oscillating movement of slug in a microchannel have been applied to the velocity solutions derived in section 7.3, and found to be producing chaotic advection. As described above in the section 7.4.1, wall velocity boundary conditions applied on the walls of the slug is determined by the

Figure 7.11. (a-f) Comparison of the effect of aspect ratio on chaotic advection for slug velocity between $\omega = \pi$ and $\pi/6$ through varianceFor example index

Figure 7.12. (a-f) Comparison of the effect of aspect ratio on chaotic advection for slug velocity between $\omega = \pi$ and $\pi/6$ through entropy index

Figure 7.13. *Wall boundary conditions for a slug moving in an oscillating motion in a meandering channel*

channel geometry in which it is moving as seen in Fig. 7.5.

7.8.1. Boundary conditions for oscillating movement of a slug

Fig. 7.13 shows the boundary conditions for an oscillating movement of a slug in a meandering channel. In a meandering channel, the slug experiences a lesser wall velocity in the inner wall and a greater wall velocity in the outer wall as seen in Fig. 7.5(a). In an oscillating motion, the slug starts from zero velocity and then accelerates to reach the maximum and then decelerates to reach zero velocity for forward motion; for the backward motion the same sequence happens — starting from zero velocity, and accelerating to reach the maximum and coming back to zero — but because the boundary conditions experienced by the slug is in the Lagrangian frame of reference, the magnitude of the velocity will be negative, as seen in Fig. 7.13. To evaluate whether this particular boundary conditions produce chaotic advection, mixing characterization by means of dye advection patterns are constructed as follows.

Figure 7.14. (a-j) *Dye advection pattern for the slug aspect ratio $\beta=2$ and droplet velocity $\pi/2$ for 20 periods*

7.8.2. Construction of dye advection patterns

Advection pattern of massless passive tracers are obtained by tracking the positions of 20,000 material points which are initially concentrated in a rectangular box of size 0.05×0.05 centered at the point (1.45, 0.45). Blue and red colour particles are used to distinguish the upper and lower half particles in the rectangular box and it is useful to visually identify the globally chaotic flow. In this study, 40 periods of meandering channel boundary conditions are used to track the particles in the slug. The maximum angular velocity (ω) attained by the slug, in the forward and the backward motion in the meandering channel, is considered from the value of $\pi/6$, $\pi/5$, $\pi/4$, $\pi/3$, $\pi/2$ and π radians/sec. Particle positions were recorded at the end of each period of tracking to see the particle advection patterns. Figures 7.14(a-j) show the dye advection patterns for the slug of aspect ratio $\beta=2$ for 20 periods.

7.8.3. Mixing characterization

Figures 7.15(a) and 7.15(b) show the 'Variance Index' and 'Shannon Entropy Index' for the dye advection patterns, as seen in Fig. 7.14. Both mixing measures show that the mixing is complete and approach the state of 'Complete Spatial Randomness' very quickly within 20 periods. Therefore, it is clear that the oscillating motion of droplet in a meandering channel geometry produces chaotic mixing and can be used for droplet and slug micromixing in microfluidic devices.

7.9. Summary

A simple 2D analytical model for evaluating and predicting chaotic advection in slug flow in high aspect ratio microchannels has been proposed. Boundary conditions mimicking the motion of the slugs in straight channel and meandering channels have been applied to get the velocity flow fields in the slugs. Poincaré maps and dye advection patterns were constructed and analysed using the statistical tools such as 'Variance Index', 'Shannon Entropy Index' and 'Complete Spatial Randomness'. It is understood that the smaller aspect ratio slugs need higher operating velocity and the intermediate aspect ratio slugs experience better mixing independent of the velocity of the droplet and the higher aspect ratio slugs tend to produce lower rate of chaotic advection irrespective of the velocity. Therefore, it is clear that the aspect ratio and velocity can play a vital role and by changing these two prudently, according to

Figure 7.15. Comparison of variance index I_{var} (a) and Shannon entropy index (b) for $\beta = 2$ for the slug angular velocity from the value of π , $\pi/2$, $\pi/3$, $\pi/4$, $\pi/5$ and $\pi/6$ radians/sec for 20 periods for the dye advection pattern in Fig. 7.14

the operating conditions and requirements, can lead to an efficient operation of slug and plug micromixing. A new boundary condition has been found to produce chaotic advection in microfluidic slugs which can be further investigated to find new geometries.

8. Conclusions and Future Research Directions

8.1. Conclusions

This thesis deals with three aspects of droplet microfluidics: droplet formation, reagent addition to droplets and droplet micromixing.

8.1.1. Droplet formation in a T-junction

In chapter 4, the findings of a numerical investigation on the mechanism of droplet break-up in a microfluidic T-junction in terms of pressure in both the continuous phase and the dispersed phase were presented. The numerical flow visualisation of the droplet break-up mechanism was validated with the experimental flow visualisation. From the computational results it was shown that the pressure profile of the dispersed phase and the continuous phase in the squeezing regime changes as the droplet break-up process proceeds. The dispersed phase pressure profile changes along with the continuous phase pressure and does not remain constant as it has been assumed by other researchers. New insights on the pressure difference between the dispersed phase and the continuous phase during the droplet break-up process is provided and we show that the minimum pressure difference happens at the moment of the droplet break-up and not during the second and third stage of the droplet formation mechanism in the squeezing regime as suggested by other researchers.

8.1.2. Reagent addition to droplets

In chapter 5, experimental results of reagent addition to microfluidic droplets was presented. In this chapter, a design was proposed that reliably adds reagents into droplets by exploiting the physics of fluid flow at a T-junction in the microchannel and the suitability of the design

for practical microfluidic system was demonstrated. An expanded section right after the T-junction enhances merging of a stream with a droplet, eliminates the drawbacks such as extra droplet formation and long mixing time. The expanded section reduces the pressure build-up at the T-junction and minimizes the tendency to form extra droplets; plays the role in creating low Laplace pressure jump across the interface of the droplet forming from the T-junction which reduces the probability of forming extra droplet in the merging process; provides space for droplet coalescence if there is an extra droplet due to droplet break-up before merging. In this design, after merging, the reactants are in axial arrangement inside the droplets which leads to faster mixing. Reliable addition of reagent to the droplets happens for the combination of flow rates in a broad range from 25 $\mu\text{l/hr}$ to 250 $\mu\text{l/hr}$, for both DI water (Q_{DI}) and fluorescent (Q_{fluor}) streams. Addition of reagents to droplets has been numerically simulated using the VOF model and compared with the experimental flow visualizations. After validating the computational flow visualization results against the experimental results, the flow field and the pressure measurements have been used to describe the physics of fluid flow responsible for reliable operation of reagent addition to droplets.

8.1.3. A model for evaluating and predicting droplet micromixing

A simple method for evaluating chaotic advection in slug micromixing was reported in this chapter. A slug moving in a slit microchannel ($(w \gg h)$) and flow field in a plane far from the boundary walls was modelled as two-dimensional low-Reynolds-number flow (Stokes flow). The two-dimensional analytical solution is compared with a two-dimensional slice from the three-dimensional numerical solution of the slug velocity field. Boundary conditions mimicking the motion of the slugs in microchannel geometries, in Lagrangian frame of reference, is used to track the passive tracer particles using Lagrangian particle tracking method. Poincaré sections and dye advection patterns are used to analyse chaotic advection of passive tracer particles using statistical concepts such as ‘Variance’, ‘Shannon entropy’ and ‘Complete spatial randomness’. An optimization exercise has been carried out to find the optimal operating variables for faster mixing in the meandering microchannel. A method for finding new channel geometries which enhance chaotic mixing was also proposed. This method has been applied to find new channel geometry, which enhance chaotic mixing in microfluidic droplets.

8.2. Further Research Directions

While a significant amount of new knowledge about the design and operation of droplet-based microfluidic system has been generated in this research, there is much more scope to develop new methodologies based on the current findings. For instance, new geometry and the microchannel designs for adding reagents to droplets can be further developed that would result in a reliable reagent addition operation.

Another interesting aspect would be to use the results obtained for the oscillating motion of a slug in a meandering channel to develop new microchannel geometries that could address the space constraint for mixing in microfluidic devices.

Following the oscillating droplet micromixing design, which has been found by the application of the method explained in chapter 7, new geometries for promoting chaotic advection in microfluidic droplets can be developed.

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A. Appendix

A.1. 2D modelling of velocity field inside a slug

For the Stokes flow in the slug, the governing equation is biharmonic

$$\nabla^4 \varphi = 0 \quad (\text{A.1})$$

where φ is the stream function, which is defined as

$$u_x = \frac{\partial \varphi}{\partial z}; \quad u_z = -\frac{\partial \varphi}{\partial x} \quad (\text{A.2})$$

This definition of φ satisfies the continuity equation automatically. The stream function is constant on the boundaries. Here, we set it to be zero.

$$\varphi(0, z) = 0; \quad \varphi(L, z) = 0; \quad \varphi(x, 0) = 0; \quad \varphi(x, h) = 0 \quad (\text{A.3})$$

The two ends of the slug are considered as free surfaces, because the viscous of the air is neglected. The boundary conditions at these two ends are respectively

$$\frac{\partial^2}{\partial x^2} \varphi(0, z) = 0; \quad \frac{\partial^2}{\partial z^2} \varphi(L, z) = 0 \quad (\text{A.4})$$

The velocities at the outer and the inner sides walls are $-S'$ and $-S$ respectively

$$\frac{\partial}{\partial z} \varphi(x, 0) = -S'; \quad \frac{\partial}{\partial z} \varphi(x, h) = -S \quad (\text{A.5})$$

Nondimensionalization

Using the height of the channel h and the velocity of the wall V_s to nondimensionalize x, z, φ

$$\hat{x} \equiv x/h; \quad \hat{z} \equiv z/h; \quad \hat{u}_x \equiv u_x/V_s; \quad \hat{u}_z \equiv u_z/V_s; \quad \hat{\varphi}_z \equiv \varphi/(hV_s) \quad (\text{A.6})$$

The dimensionless governing equation is

$$\left(\frac{\partial^4}{\partial \hat{x}^4} + 2 \frac{\partial^4}{\partial \hat{x}^2 \partial \hat{z}^2} + \frac{\partial^4}{\partial \hat{z}^4} \right) \hat{\varphi} = 0 \quad (\text{A.7})$$

and the dimensionless boundary conditions are respectively

$$\hat{\varphi}(0, \hat{z}) = 0; \quad \hat{\varphi}(\beta, \hat{z}) = 0 \quad (\text{A.8})$$

$$\hat{\varphi}(\hat{x}, 0) = 0; \quad \hat{\varphi}(\hat{x}, 1) = 0; \quad \frac{\partial^2}{\partial \hat{x}^2} \hat{\varphi}(0, \hat{z}) = 0; \quad \frac{\partial^2}{\partial \hat{x}^2} \hat{\varphi}(\beta, \hat{z}) = 0 \quad (\text{A.9})$$

$$\frac{\partial}{\partial \hat{z}} \hat{\varphi}(\hat{x}, 0) = -\eta; \quad \frac{\partial}{\partial \hat{z}} \hat{\varphi}(\hat{x}, 1) = -\xi \quad (\text{A.10})$$

where $\beta \equiv L/h$ is the aspect ratio; $\eta \equiv S'/V_s$ and $\xi \equiv S/V_s$ are dimensionless velocities at the walls of the channel.

Analytical solution

Using the Finite Fourier Transform (FFT) method, the solution of the biharmonic equation can be written as

$$\hat{\varphi}(\hat{x}, \hat{z}) = \sum_{n=0}^{\infty} [\varphi_n(\hat{z}) \sin(\alpha_n \hat{x}) + \psi_n(\hat{z}) \cos(\alpha_n \hat{x})] \quad (\text{A.11})$$

which is the Fourier transform of $\hat{\varphi}(\hat{x}, \hat{z})$, where $\alpha_n = \frac{n\pi}{\beta}$. According to the boundary conditions A.9, the solution can be written in a simpler format

$$\hat{\varphi}(\hat{x}, \hat{z}) = \sum_{n=1}^{\infty} \varphi_n(\hat{z}) \sin(\alpha_n \hat{x}) \quad (\text{A.12})$$

Using the FFT method, the dimensionless stream function φ can be determined as

$$\begin{aligned}
\hat{\varphi}(\hat{x}, \hat{z}) &= \sum_{n=1}^{\infty} \sin(\alpha_n \hat{x}) \times \\
&(C_{1n} \cosh(\alpha_n \hat{z}) + C_{2n} \sinh(\alpha_n \hat{z}) \\
&+ C_{3n} \hat{z} \cosh(\alpha_n \hat{z}) + C_{4n} \hat{z} \sinh(\alpha_n \hat{z}))
\end{aligned} \tag{A.13}$$

The dimensionless velocity components in the $\hat{x}$ and $\hat{z}$ directions are respectively,

$$\begin{aligned}
\hat{u}_x &= \frac{\partial}{\partial \hat{z}} \hat{\varphi}(\hat{x}, \hat{z}) = \sum_{n=1}^{\infty} \sin(\alpha_n \hat{x}) \\
&\times [C_{1n} \alpha_n \sinh(\alpha_n \hat{z}) + C_{2n} \alpha_n \cosh(\alpha_n \hat{z}) + C_{3n} \alpha_n \hat{z} \sinh(\alpha_n \hat{z}) \\
&+ C_{3n} \cosh(\alpha_n \hat{z}) + C_{4n} \alpha_n \hat{z} \cosh(\alpha_n \hat{z}) + C_{4n} \sinh(\alpha_n \hat{z})]
\end{aligned} \tag{A.14}$$

$$\begin{aligned}
\hat{u}_z &= -\frac{\partial}{\partial \hat{x}} \hat{\varphi}(\hat{x}, \hat{z}) = -\sum_{n=1}^{\infty} \cos(\alpha_n \hat{x}) \times \\
&(C_{1n} \cosh(\alpha_n \hat{z}) + C_{2n} \sinh(\alpha_n \hat{z}) \\
&+ C_{3n} \hat{z} \cosh(\alpha_n \hat{z}) + C_{4n} \hat{z} \sinh(\alpha_n \hat{z}))
\end{aligned} \tag{A.15}$$

The constant coefficients can be determined from the boundary conditions Eq. A.8 and Eq. A.10,

$$C_{1n} = 0 \tag{A.16}$$

$$C_{2n} = -\frac{4\xi}{D_n \beta \alpha_n} \sinh(\alpha_n) + \frac{4\eta}{D_n \beta} [\sinh^2(\alpha_n) - \cosh^2(\alpha_n)] \tag{A.17}$$

$$C_{3n} = \frac{4\xi}{D_n \beta} \sinh(\alpha_n) + \frac{4\eta \sinh^2(\alpha_n)}{\beta \alpha_n D_n} \tag{A.18}$$

$$\begin{aligned}
C_{4n} &= \frac{4\xi}{D_n\beta\alpha_n} [-\alpha_n \cosh(\alpha_n) + \sinh(\alpha_n)] \\
&+ \frac{4\xi}{D_n\beta\alpha_n} [-\sinh(\alpha_n) \cosh(\alpha_n) - \alpha_n \sinh^2(\alpha_n) + \alpha_n \cosh^2(\alpha_n)]
\end{aligned} \tag{A.19}$$

where,

$$D_n = \alpha_n^2 \cosh^2(\alpha_n) - (\alpha_n^2 + 1) \sinh^2(\alpha_n) \tag{A.20}$$