Electromigration and micromixing through a constriction under time periodic electroosmotic flow

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**ELECTROMIGRATION AND MICROMIXING THROUGH A CONSTRICTION
UNDER TIME PERIODIC ELECTROOSMOTIC FLOW**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

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Abstract

Microfluidic devices have found numerous applications in chemical analyses and biomedical diagnoses. A micromixer is often an integral component in these devices where rapid mixing of two or more fluids in short distance is required. Mixing in a microchannel is a challenging task due to the small Reynolds numbers and lack of turbulence. Various micromixing techniques have been proposed and demonstrated over the past 10 years. Current methods for improving mixing in microchannels often involve complicated geometries, long channels, embedded metallic structures or very high voltage. These features might induce additional cost and complication in the integration of micromixers into various microfluidic devices.

A simple yet an efficient novel approach to enhance mixing has been proposed in this thesis. The concept was demonstrated in a T-type micromixer with a constriction introduced at the T-junction under time periodic electroosmotic flow. Two sinusoidal AC electric fields with 180° phase difference and similar DC bias were applied at the two inlets. The two out-of-phase AC electric fields induced oscillation of fluid interface at the junction of the two inlet channels and the constriction. Fluids from these two inlets formed alternative plugs in the constricted channel. These plugs of fluids radiated downstream from the constriction into the larger channel and formed alternate thin crescent-shaped layers of fluids. These crescent-shaped layers of fluids increased the contact surface between the two streams of fluids significantly and thus enhanced mixing efficiency. Experimental results and theoretical analyses revealed that factors governing the mixing efficiency include amplitude and frequency of the AC electric fields, and the length and location of the constriction.

Numerical analyses of the proposed micromixer were conducted with finite element method (FEM) using COMSOL Multiphysics software. The validity and accuracy of the numerical model developed were verified experimentally. The model allows the prediction of operating parameters such

as AC amplitude and frequency for optimum mixing. In most micromixing investigation, fluorescent are often employed to represent the solutes to be mixed and facilitate mixing efficiency evaluation. However, most researchers employ neutral dyes in their experimental investigation or assume that the fluorescent dye is neutral despite the abundance of charge solutes. It is discovered that for an accurate simulation and representation of flow behavior and mixing condition in a practical micromixer, the often neglected electromigration effect of charged fluorescent dye or solute must be considered. In view of the importance of the electromigration effect, a convenient method to measure ionic mobility of the fluorescent dye has been adapted and developed from the micromixer design.

Important factors that govern the mixing efficiency of the mixer, such as the contact area between the two fluids and the amplitude of fluid interface oscillation were quantified based on experimental and numerical results. The effect of phase difference between the applied electric fields on mixing efficiency was also investigated through the numerical simulations. Detailed error analyses were performed to examine the sensitivity of simulation results to the variability of constriction width, diffusion coefficient, and ion mobility.

Numerical simulations of electroosmotic flow in micromixers often require zeta potential to be specified as the boundary condition. One of the common methods to measure zeta potential is current monitoring method, which involves one fluid displacing another fluid with small concentration difference. There is experimentally observed anomaly in such flow: the displacement time for a high concentration solution displacing a low concentration solution is smaller than the displacement time in the reverse direction. The percentage displacement time difference increases with increasing concentration difference and is independent of the length or width of the channel and the voltage applied. Hitherto, no theoretical analysis or numerical simulation has been conducted to explain this phenomenon. To describe this phenomenon, a new formulation which can model the continuous change in zeta potential has been proposed. Numerical simulations based on this new formulation were

performed to explain the experimental observations and to elucidate the mechanisms involved. Simulations showed that the velocity profile and ion distribution deviate significantly from a single fluid electroosmotic flow. The distortion of ion distribution near the electrical double layer (EDL) is responsible for this displacement time difference for the two different flow directions. The general trends obtained from numerical studies agree with the experimental findings.

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List of Symbols

<u>Symbol</u>	<u>Units</u>	<u>Description</u>
c	M	concentration of neutral species
c_o	M	bulk concentration
c_i	M	concentration of ionic (charged) species
$c_{\pm}$	M	concentration of positive/negative ion
c_p	Jkg ⁻¹ K ⁻¹	specific heat
c_i^*	-	dimensionless concentration
$\bar{c}_i$	-	normalized concentration at point of evaluation
$c_{\pm}$	M	concentration of positive / negative ion
$\bar{c}_{0i}$	-	normalized concentration at point of evaluation with no mixing
$\bar{c}_{\infty}$	-	normalized concentration at point of evaluation with complete mixing
e	C	unit electron charge
f	s ⁻¹	frequency
i	A	current density
k_b	m ² kg s ⁻² K ⁻¹	Boltzmann constant
n	-	number of sample
$\mathbf{n}$	-	unit normal vector normal to boundary
p	Pa	pressure
p^*	-	dimensionless pressure
r	m	radius of semi-circular pattern formed after constriction
s_{LH}	s	sample standard deviation of T_{LH}
s_{HL}	s	sample standard deviation of T_{HL}
t	s	time
t^*	-	dimensionless time
t_s	-	t-score
$\mathbf{u}$	ms ⁻¹	fluid velocity vector
$\mathbf{u}^*$	-	dimensionless velocity
u_{av}	ms ⁻¹	average x-velocity
u_{mi}	m ² V ⁻¹ s ⁻¹	ionic mobility
$u_{m\pm}$	m ² V ⁻¹ s ⁻¹	ionic mobility of positive / negative ion
v	ms ⁻¹	flow velocity
v_{ep}	ms ⁻¹	electrophoretic velocity
v_{eo}	ms ⁻¹	slip velocity of the electroosmotic flow

v_m	ms^{-1}	electromigration velocity
w	m	width of constriction
x	m	displacement
$\mathbf{x}^*$	-	dimensionless displacement
$z_{\pm}$	-	ion charge number of positive / negative ion
A	m	amplitude of fluid interface oscillation
A_c	m^2	cross section area of channel
$A_{\text{trapezium}}$	m^2	area of trapezoidal plugs formed in constriction
D	m^2s^{-1}	diffusion coefficient
$D_{\pm}$	m^2s^{-1}	diffusion coefficient of positive/ negative ion
E	Vcm^{-1}	electric field
E_{AC}	Vcm^{-1}	amplitude of AC electric field
E_{DC}	Vcm^{-1}	magnitude of DC electric field
E_g	J	Energy generated
E_{st}	J	Energy stored
F	Cmol^{-1}	Faraday constant
$\mathbf{F}_e$	N	external body force
I_i	-	pixel intensity at point of evaluation
I_{max}	-	maximum image intensity
I_{min}	-	minimum image intensity
$\bar{I}_i$	-	normalized pixel intensity at point of evaluation
$\bar{I}_{0i}$	-	normalized concentration at point of evaluation with no mixing
$\bar{I}_{\infty}$	-	normalized concentration at point of evaluation with complete mixing
L	m	Length of channel
L_c	m	Length of contact
N	-	number of points taken in mixing evaluation
N_A	mol^{-1}	Avogadro constant
Pe	-	Péclet number
Q	m^3s^{-1}	flow rate
R	m	volume to surface ratio
R_c	-	concentration ratio between two solutions
R_L	Ωm	Resistivity of liquid
Re	-	Reynolds number
S	-	ratio of length of slanted portion to length of trapezoidal plug
St	-	Strouhal number
T	K	temperature

T_{HL}	s	displacement time for high concentration solution displacing low concentration solution
T_{LH}	s	displacement time for low concentration solution displacing high concentration solution
T_{os}	s	period of fluid interface oscillation
$\bar{T}_{LH}$	s	sample mean of T_{LH}
$\bar{T}_{HL}$	s	sample mean of T_{HL}
U	ms^{-1}	reference velocity
V	V	applied voltage(experimental)
V_c	m^3	volume of fluid
V_{AC}	V	amplitude of applied AC electric potential (experimental)
V_{DC}	V	applied DC electric potential (experimental)
W	m	width of main channel
X	m	magnitude of fluid interface displacement
α	-	ratio of electromigrative transport to convective transport.
ϵ_0	Fm^{-1}	permittivity of free space
ϵ_r	-	dielectric constant of fluid
ζ	V	zeta potential
λ_D	m	Debye length
μ	$\text{kgm}^{-1}\text{s}^{-1}$	dynamic viscosity of fluid
ρ	kgm^{-3}	density
ρ_e	Cm^{-3}	net charge density
σ	Sm^{-1}	conductivity
σ_M	-	Mixing efficiency
σ_w	Cm^{-2}	wall surface charge density
ϕ^*	-	dimensionless applied potential
ϕ_{AC}	V	amplitude of applied AC electric potential (numerical)
ϕ_{DC}	V	applied DC electric potential (numerical)
ϕ	V	applied electric potential (numerical)
ψ	V	wall electric potential
ω	s^{-1}	angular frequency
Δt	s	total time voltage is applied
ΔT	K	temperature change of liquid
∇	-	del operator

Chapter 1 Introduction

1.1 Introduction

Micro-Electro Mechanical System (MEMS) refers to a miniature system, typically on a millimeter scale, which combines mechanical, electrical, fluidic and optical components to perform certain functions, such as sensing and actuation. MEMS devices are fabricated with the same technology as the Integration Circuit (IC) industry, like deposition, etching and lithography. The early development of MEMS began in the 1970's, with commercialization in the 1990's.

In recent years, MEMS devices have found numerous applications in chemical analysis and biomedical diagnosis. Hence, BioMEMS, lab-on-a-chip or micro-total analysis system (μ TAS) are terms referring to MEMS devices which perform biomedical and chemical analyses. Lab-on-a-chip is basically the technology that integrates microfluidic system on a microscale chip and can be considered as the miniaturization of a laboratory to a small device. The so-called "laboratory" is created by means of channels, mixers, reservoirs, diffusion chambers, integrated electrodes, pumps, valves and others. Compared to a conventional laboratory test, BioMEMS provides several advantages including extremely low consumption of reagents and production of chemical waste, a more rapid analysis and a significant improvement in performance. The compact devices are portable and therefore allow samples to be analyzed on site, a rather impossible task for a conventional laboratory. These devices are normally cheap and disposable after use (Chang and Yang 2007).

Microfluidics involves the study of the behaviour, the precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter scale. It is generally a multidisciplinary field integrating engineering, physics, chemistry, material science and biology. Practical applications of microfluidic devices involve small volumes of fluid in microliter or nanoliter. Microfluidics emerged in the 1980s and is used in inkjet print head, DNA chips and lab-on-a-chip system.

There are two general approaches to transport fluids in microfluidic channel namely, pressure driven flow and electrokinetically driven flow. Electrokinetically driven flow or electroosmosis involves the movement of fluid confined within channel walls under the application of an electric field. This effect arises from charge accumulation which occurs naturally at the channel walls when it is placed into contact with an electrolyte. The electrostatic potential at the wall which governs the flow velocity and direction is called zeta potential. Electroosmotic flow (EOF) is extensively employed in microfluidic devices for pumping and mixing. It is preferred in certain applications because of its plug like flow profile, the ease of fabrication and integration into other electrical devices.

1.2 Research Motivation

In most MEMS devices with microfluidic elements, mixing of fluids are often required. However, owing to the small length scale involved in microfluidics, the Reynold number for flows in a microfluidic device is typically very small (below 100). Hence, mixing through turbulent flow induced by inertial/viscous effect for aqueous solutions is not possible in microfluidics. Mixing in microfluidic devices is largely attributed to diffusion process, which often requires long channel or complicated geometries. This complicates the fabrication process and increases the cost.

Current methods for improving mixing in microchannels often involve complicated geometries, long channels, embedded metallic structures or very high voltage. These features might induce

additional cost and complication in the integration of micromixers into various microfluidic devices. Therefore, it is desirable and advantageous to have a simple yet efficient method to improve mixing performance under electroosmotic flow in micro channels, a motivation of this investigation.

It is known that time periodic electric field (Chang and Yang 2007) or introducing a constriction (Brotherton *et al.* 2008) has the potential to enhance the mixing efficiency. The combined effects of constriction and sinusoidal driving electric field on mixing performance have yet to be investigated. In this investigation, a novel method which involves time periodic driving electric field and a simple constriction is proposed and demonstrated. Despite its high mixing efficiency, the proposed micromixer is one of the simplest micromixers available in the literature. It has a simple two dimensional geometry and does not require any embedded electrodes or metallic structures. The various factors governing the mixing efficiency such as the frequency and amplitude of the sinusoidal electric field, the location and length of the constriction were examined. The principle behind the mixing mechanism of the mixer was inspected through analytical and numerical analysis.

In micromixing investigation, fluorescent dye is often added to one of the fluid stream for flow visualization and mixing efficiency evaluation of micromixers. However, in most numerical simulations of flows in micromixers, the mobility of fluorescent dye is often neglected and this might induce errors in the prediction of the observed flow behaviour. Hence, this investigation examined the effect of fluorescent dye mobility in the flow simulation of electrokinetically driven micromixers.

Zeta potential is an important parameter which governs the flow velocity and direction of electroosmotic flow. The value of zeta potential is required in the numerical simulation of electroosmotic flow in micromixer. One of the popular method to determine zeta potential is current monitoring technique, which involves the observation of current response as a fluid displaces another fluid with a slight difference in concentration in a microchannel. The

displacement time required for a fluid to fully displace another fluid which resides in the microchannel initially can be determined from the current response and related to the zeta potential. However, there is experimentally observed anomaly in such flow, namely the displacement time is flow direction dependent; it has been observed that the time required for a low concentration solution to displace a high concentration is not the same as the time required for a high concentration solution to displace a low concentration solution. Hitherto, no theoretical or numerical studies have been conducted to explain this phenomenon. Experimental and numerical analyses are performed in this investigation to verify and explain this interesting phenomenon. The understanding of this phenomenon is important because two fluid displacement flows are commonly encountered in various microfluidic systems, such as micromixers and current monitoring experiment.

1.3 Outline of Thesis

Chapter 2 reviews the development of various micromixers with electrokinetically driven mechanism and/or enhances mixing through a constriction.

Chapter 3 describes the concept and mechanism of the proposed micromixer. This chapter identifies and describes the two distinct phases in the mixing enhancement process, and reveals the various factors governing the mixing efficiency through a preliminary analytical model.

Chapter 4 documents the fabrication of the proposed micromixer with soft lithography technique and the experimental setup for mixing enhancement investigation through fluorescent microscopy. The various designs of the micromixer for the understanding of the characteristics of the micromixer are described here.

Chapter 5 presents the experimental results for the mixing efficiency of the proposed micromixer. Various factors which govern the mixing efficiency are identified and investigated.

Chapter 6 describes the experimental methodology to obtain zeta potential and fluorescent dye mobility, which are necessary to model the flow in Chapter 7.

Chapter 7 presents the numerical studies on the electroosmotic flow in the micromixer. Simulation are performed with COMSOL multiphysics. The importance of including fluorescent dye mobility in micromixing simulation under electroosmotic flow is illustrated in this chapter.

Chapter 8 describes the experimental studies on the anomaly observed in two-fluid displacement electroosmotic flow, whereby the displacement time is direction dependant. A numerical model is proposed to describe the mechanism behind this phenomenon.

Chapter 9 summarizes and reiterates the contributions of the investigation contained in this thesis.

Chapter 2 Review of Electroosmotic Flow and Micromixing

2.1 Introduction

Electroosmotic flow is one of the common methods to transport fluids in microchannels. This chapter introduces the basic theories on electrokinetic phenomena and electroosmotic flow in microfluidic channels. Mixing two or more fluids is essential in many microfluidic devices. However, mixing in microfluidic channels is difficult due to the lack of turbulence in these small channels. Research and development of various efficient micromixers have been conducted over the recent years. Various micromixing schemes under electroosmotic flow will be reviewed in this chapter. Two important aspects in the simulation of an electrokinetically driven micromixer, namely ionic mobility of fluorescent dye and zeta potential of microchannel will also be introduced in this chapter.

2.2 Electrical Double Layer (EDL)

When certain solid is in contact with a liquid, surface charge is developed at the interface. There are a few mechanisms of charge development at the interface; some examples are difference in the affinity of the two phases for electrons or ions, ionization of surface groups and physical entrapment of non-mobile charge in one phase (Hunter 1988). These processes lead to the build-up of an electric charge at the solid surface, which creates an electrostatic field that affects the ions distribution in the liquid

phase. The separation of charge that occurs at the interface between the solid and fluid phase is called an electrical double layer because it consists ideally, of two regions of opposite charge.

The simplest model of such a system assumes that both charge layers are fixed in parallel planes. However, this assumption is not correct in a liquid phase where ions experienced both electric and thermal diffusive forces. Gouy (1910) and Chapman (1913) independently proposed a diffuse model of the electrical double layer, in which the potential at a surface decreases exponentially due to the adsorbed counter-ions (ions with the opposite charge to the surface) from the solution (Hunter 1988), as shown in Figure 2.1. In current classical theory, the region where the immobile counter ions is adsorbed to the solid surface is called the Stern layer whereas the Gouy-Chapman diffuse layer consists of the mobile and diffuse counter ions. This diffuse layer has a non-zero net charge because of the high concentration of counter ions as compared to the co-ions (ions with the same charge to the surface). It can move under the influence of a tangential force when an electrical field is applied.

Figure 2.1: Electric double layer (EDL)

This diffuse layer screens the electric surface charge at the solid surface. The net electric charge in this screening diffuse layer is equal in magnitude to the net surface charge, but has the opposite polarity. Therefore, the bulk region which is far from the solid surface is electrically neutral. Conventionally, a slipping plane is conceptually introduced which separates the mobile fluid from the fluid that remains attached to the surface. Electrical potential at this plane is called the zeta potential or ζ -potential. The characteristic thickness of the double layer is the Debye length λ_D . For an aqueous solution it is in the order of nanometers.

2.3 Electrokinetic Phenomena

The formation of EDL gives rise of various electrokinetic phenomena. There are basically 4 types of electrokinetic phenomena (Probstein 1994):

a) Electroosmosis: the movement of a liquid relative to a stationary charged surface (for example a microchannel or porous media) by an applied electric field. For example, when the capillary tube is negatively charged, the applied electric field exerts a force on the ions in the diffuse layers, which has an excess of positively charged ions. The positive ions will move towards the cathode, and drag the bulk of the electrolyte along with it. For a thin EDL, the slip velocity of the electroosmotic flow, v_{eo} is commonly given by the Helmholtz-Smoluchowski equation,

$$v_{eo} = \frac{-\epsilon_r \epsilon_0 E \zeta}{\mu} \quad (2.1)$$

where ϵ_0 = permittivity of free space, ϵ_r = dielectric constant of fluid, E = electric field, ζ = zeta potential and μ = dynamic viscosity of fluid. The term $\frac{\epsilon_r \epsilon_0 \zeta}{\mu}$ is often grouped together and termed as the electroosmotic mobility.

b) Electrophoresis: the movement of a charged particle or surface relative to a stationary liquid by an applied electric field. A typical example of electrophoresis involves a negatively charge colloidal particle migrating towards the anode in a solution. For a very small colloidal particle with diameter much smaller than the Debye length, the particle can be considered as a point charge and the electrophoresis velocity is given by,

$$v_{ep} = \frac{2\varepsilon_r\varepsilon_0 E\zeta}{3\mu} \quad (2.2)$$

When the diameter of a charged particle is large as compared to the Debye length, the EDL at the particle surface can be approximated as that for a flat plate. The electrophoresis velocity can then be given by,

$$v_{ep} = \frac{\varepsilon_r\varepsilon_0 E\zeta}{\mu} \quad (2.3)$$

Note that the formulae for electrophoresis appears similar to that of electroosmosis, with opposite sign. Therefore, electrophoresis and electroosmosis can be considered as compliments of each other.

c) Streaming potential: the electric field created when a liquid is made to flow along a stationary charged surface.

d) Sedimentation potential: the electric field created when charge particles are sedimenting through a stationary liquid.

2.4 Electroosmotic Flow in Microchannel

Flow in microchannel can be generated by applying either a pressure or an electric field. In a pressure driven system, an external pump provides the driving force. Pressure driven flow generally shows a

parabolic velocity flow profile (see Figure 2.2). Velocity is fastest at the centre and slows down to zero at the boundaries at the wall. For an analytical tool, parabolic flow profile might be undesirable as it causes band broadening. Furthermore, conventional pumps must generate high pressures for delivering fluids in microchannels with small diameters.

Figure 2.2: Flow profile of electroosmotic flow and pressure-driven flow

In contrast, electroosmotic flow has a unique plug-like velocity flow profile (Figure 2.2). The flow velocity increases rapidly from zero at the wall to a maximum at the slip plane and then remains constant across the whole cross section area. It is desirable to have a uniform flow profile in an analytical tool due to the absence of band broadening. Furthermore, the flow velocity can be controlled accurately by adjusting the strength of the electric field.

Electroosmotic flow occurs in microchannel when an electric field is applied across it. For glass or fused silica, a material commonly used for microfluidic systems, the silanol groups (SiOH) of the inner

surface of the channel will deprotonate at $\text{pH} > 2$ when in contact with an electrolyte (Hunter 1988). Deprotonation is the removal of a proton from a molecule, forming the conjugate base. In this case, deprotonation of SiOH results in the production of SiO^- and H^+ . This causes a negative surface charge (due to the presence of SiO^-) and positive ions in the electrolyte are attracted to the surface. When an electric field is applied, an electric force would be exerted on the ions in the diffused layer, thus causing a net motion of the positive ions in the direction of the electric field. These moving ions induce a bulk fluid flow due to the viscous properties of fluid, causing electroosmotic flow.

Polymer-based microchannels have been used extensively in microfluidic research in recent years. Among them, polydimethylsilanol (PDMS) is a popular material due to its optical transparency, ease of fabrication and good sealing properties (Duffy *et al.* 1999). It is generally known that native PDMS has less ionizable surface sites than glass or fused silica. Thus it exhibits a slower electroosmotic flow towards the cathode. PDMS is often bonded to glass through oxygen plasma treatment to produce a PDMS/Glass hybrid device. It has some desirable properties over a pure PDMS device, such as better wettability (Bhattacharya *et al.* 2005) (which facilitates filling of solution into the microchannel) and low temperature rise (Erickson *et al.* 2003) from Joule heating (as glass surface dissipates heat quickly).

2.5 Dimensionless Numbers

Dimensionless numbers in the thesis are defined in the following sub-sections.

2.5.1 Reynolds Number

Reynolds number is defined as the ratio of inertial force to viscous force and it is often given by,

$$\text{Re} = \frac{\rho v L}{\mu} \quad (2.4)$$

where ρ = density of fluid, v = flow velocity, L = characteristic length, and μ = dynamic fluid viscosity.

2.5.2 Péclet Number

Péclet number is defined as the ratio of the rate of convective transport to diffusive transport of mass, and it is given by,

$$Pe = \frac{vL}{D} \quad (2.5)$$

where D = diffusion coefficient.

2.5.3 Strouhal Number

Strouhal number represents the ratio of the characteristic time period of the flow to the forcing (pulsing) period. It is useful for analyzing oscillating fluid flow dynamics and is given by,

$$St = \frac{fL}{v} \quad (2.6)$$

where f = oscillation frequency.

2.6 Mixing in Microchannel

Mixing of two or more miscible fluids in microfluidic systems is essential, for example to achieve a homogenized solution of various reagents to ensure maximum products in chemical reactions. An ideal micromixer should possess features of rapid and efficient mixing, be compact in size, and be easily integrated with other components, and can be fabricated cheaply and easily. Achieving such a mixer is an essential but challenging task when developing microfluidic devices for chemical and biological analysis applications.

Since viscous forces dominate the flow in microfluidic devices, and the flow is limited to low Reynolds number regime, turbulence does not readily occur for aqueous solutions, as opposed to macro flow. In channel with electroosmotic flow, the Reynold number is typically less than unity, which is in the Stoke flow regime. Due to a lack of inertial effect, mixing of two fluid streams within a microchannel is largely attributed to the diffusion process. However, the rate of diffusive mixing in micro channel is very slow as compared to the convection of fluid down the channel, because the Péclet number of a typical micro channel flow is very high.

Although it is difficult to induce turbulence (so-called Eulerian chaos) in microchannels, effective mixing in low Reynolds number flow can be obtained by a chaotic advection mechanism (or so-called Lagrangian chaos or laminar chaos). This mechanism provides an effective increase in the interfacial contact area and concentration gradient due to the reduction of diffusion length(Wiggins and Ottino 2004). However, chaotic advection cannot occur in steady two-dimensional flows, and is restricted to two-dimensional time dependant flows and three-dimensional flows. For example, a small cylindrical rotor spinning around its centre with a uniform angular frequency (Couette flow) is probably not an efficient mixer, while two such rotors turned on and off alternatively (a blinking vortex model) can generate effective mixing (Ottino 1989). In Couette flow, the fluid is only stretched and chaotic advection cannot be induced. In contrast, when two alternating rotors are operating together, the fluid is repeatedly stretched around one of them and then folded around the other, thus generating chaotic advection. Therefore, effective mixing in micro channel with laminar flow always required repeated stretching and folding of fluid elements.

2.7 Micro Mixing based on Electrokinetics

Electrokinetic driven flow has many advantages for manipulating fluids in a microfluidic device. Unlike pressure driven flow with a syringe pump, with the associated difficulty of transporting fluids through leaking connectors, electrokinetic pumping can be integrated easily into microdevices with external or embedded electrodes, without worrying about leakage. In addition, electrokinetic pumping requires no external mechanical moving parts. Such system can be made portable by replacing the power supply with a battery (Chang and Yang 2007).

In electroosmotic flow, an electric field is often used not just to transport the fluid, but also to enhance mixing. Electrokinetic mixing can be broadly categorized as either passive or active mixing (Chang and Yang 2007). Passive mixing refers to mixing enhanced by virtue of their particular geometry topologies, surface properties, or instability phenomenon which occurs naturally under a static electric field. Active mixing refers to the enhancement of mixing in electrokinetically driven microfluidic systems with a time-dependant electric field or a mechanical stirring mechanism. Some micro-mixing schemes based on electrokinetics are described in the following sub-sections.

2.7.1 Electrokinetic Instability (EKI)

Electrokinetic instability (EKI) phenomenon in microchannel was first investigated experimentally by Oddy *et al* (2001). This instability is caused by the charge accumulation at the interface of two fluids with different electrical conductivity (Chen *et al.* 2005). Flow instabilities can be generated if high voltage is applied to the two fluids with large conductivity difference (typically at a ratio of 10:1).

Two micromixers have been implemented by Oddy *et al* (2001) based on EKI effect (see Figure 2.3). In the first micromixer (width = 1mm, depth = 300 μ m), AC voltage of 1kV with a frequency of 10Hz

was applied to drive and mix the fluids (see Figure 2.3a). Within 2s from the application of the electric field, the flow became unstable and transverse velocities in the flow rapidly stretched and folded the fluid interface. The flow instability serves as an effective stirring mechanism through the channel to enhancing mixing. In the second micromixer, the fluids were driven by syringe pump into a mixing chamber (1mm x 1mm x 300 μ m), where a voltage of 4kV with a frequency of 5Hz was applied. Good mixing was achieved within 3s due to the EKI induced stirring in the chamber.

Figure 2.3: EKI micromixer under (a) electroosmotic flow (b) pressure driven flow (Oddy *et al.* 2001).

Tai *et al* (2006) had presented a T-shaped micromixer featuring 45° parallelogram barriers within the mixing channel (Figure 2.4). When a DC electric field of 150V/cm was applied across the micromixer (width = 300 μ m, depth = 30 μ m), the flow was stable and mixing efficiency was low. If the electric field was increased beyond 306V/cm, the device achieved rapid mixing of the two sample fluids with a conductivity ratio of 10:1 (sample to running buffer concentration) by means of EKI shedding

effects. Mixing efficiency of 91% and 80% were achieved at 300V/cm and 500V/cm at 2.3mm downstream (7.6 times of the width of the channel).

Figure 2.4: EKI mixing in channel with parallelogram barriers. Applied electric field: (a) 150V/cm (b) 300V/cm (c) 500V/cm (Tai *et al.* 2006)

Micromixer based on EKI has also been developed by Park *et al* (2005) with various wall topology, see Figure 2.5. The conductivity ratio of the two mixing streams was also set to 10:1. Mixing performance was found to improve by the wall cavities as compared to a straight channel (width = 50 μ m, depth = 50 μ m). At an applied DC voltage of 510 V/cm, a maximum efficiency of 65% was achieved within a distance of 0.75mm (15 times of the width of the channel). Both methods by Tai *et al* and Park *et al*, which involve multiple wall protrusions or cavities, require a long distance (7.6~15 times of channel width) to achieve good mixing as the liquids closer to the wall are difficult to be unmixed by the instability effect.

Huang *et al* (2006) has investigated mixing with a cross-shape channel with an added pair of barriers, see Figure 2.6b. EKI will only happen if the electrical field strength exceeds a certain threshold value (or the critical electrical field strength) at a certain conductivity ratio. Different conductivity ratios

for the mixing streams were experimented and the corresponding critical electric field strengths were obtained. Their result is shown in Figure 2.6c.

A very high electric field is required to trigger EKI for two solutions with low conductivity ratio. The employment of a pair of barriers only lowered slightly the critical electrical field strength. Therefore, micromixing based on EKI is only applicable to two fluids with high conductivity ratio. It can be observed that the critical electric field strength approaches an asymptotic limit when the conductivity ratio exceeds 20.

Figure 2.5 : EKI mixing in channel with different types of wall cavities (Park *et al.* 2005)

Figure 2.6 : (a) Cross-shaped microchannel without barrier (b) Cross-shaped micromixer with a pair of barriers (c) Critical electric field strength for different conductivity ratio (Huang *et al.* 2006)

2.7.2 Surface Heterogeneity

Erickson and Li (2002) performed a numerical simulation on microchannel with patches of heterogeneous surfaces. The heterogeneous regions with a net surface charge density of opposite sign to that of the homogenous surface (or equivalently ζ -potential of opposite sign) can result in build up flow circulation near these patches (Ajdari 1995), see Figure 2.7a. In their numerical studies, Erickson and Li (2002) assumed the zeta potential of the heterogeneous patches and the homogenous channel to be +45mV and - 45mV respectively. Two arrangements of the heterogeneous patches were introduced at the side walls of T-channels (see Figure 2.7b,c). The staggered patches arrangement (c) was found to be

more efficient and resulted in a 50% and 30% reduction in mixing length for channels with 25 μm and 50 μm width respectively, as compared to a homogenous channel of similar dimensions.

Figure 2.7: Flow circulation at heterogeneous surface. Simulations of microchannel with (a) homogeneous surface (b) symmetrical arrangement of heterogeneous patches (c) staggered arrangement of heterogeneous patches. (Erickson and Li 2002).

Micromixers based on surface heterogeneity were implemented by Biddiss *et al* (2004), see Figure 2.8. The heterogeneous patches were fabricated at the channel floor through polyelectrolyte coating method developed by Liu *et al* (2000). With a staggered patch arrangement, mixing efficiency was improved from 36% to 96% in comparison with a homogenous channel (width = 200 μm). The mixing

length was reduced from 22mm to 2.6mm, which was a significant 88% reduction. However, in actual practice, it would be complicated and costly to fabricate microchannel with patches of heterogeneous surfaces.

Figure 2.8: Micromixer based on heterogeneous patches on channel floor (Biddiss *et al.* 2004)

2.7.3 Embedded Electrode

Besides surface heterogeneity, local recirculation of fluids can also be induced by embedded electrodes at the surfaces of microchannels. Sasaki *et al* (2006) demonstrated a micromixer with an applied DC-biased AC electric field in a transverse direction to the pressure driven fluid flow. The micromixer consists of a pair of asymmetrical planar parallel electrodes arranged along the channel floor (channel width = 120 μ m), see Figure 2.9a. A high frequency AC electric field (20V_{pp}, 1 kHz) was applied at the electrodes to generate rotating vortices above the electrode through AC electroosmosis. At $Pe < 2 \times 10^{-3}$, the time to reach 90% mixing efficiency was reduced from 4.4 sec to 0.18sec, in comparison with a diffusion-based micromixer of similar dimensions.

Alternatively, Ng *et al* (2009) applied an AC electric field with a DC offset (2.5V) across a pair of symmetrical planar electrodes to induce a net flow across the width of the channel (see Figure 2.9b). An AC signal with amplitude 15 to 25 V_{pp} and frequency of 10 kHz to 1 MHz induced large flow vortices above the planar electrodes and flow recirculation across the depth of the channel. The highest mixing efficiency achieved was 92% in a mixing length as short as 600μm (6 times the channel width) in less than 80ms with a flow rate of 2μl/min. However, mixing efficiency was lower (65%) for solution with high conductivity (500mS/m), as the amplitude of applied voltage was restricted due to electrolysis and formation of bubbles.

Figure 2.9: AC electroosmotic mixer with (a) asymmetrical planar electrodes (Sasaki *et al.* 2006) (b) symmetrical planar electrodes (Ng *et al.* 2009)

Lynn *et al* (2008) had implemented a T-type micro mixer with integrated electrodes at the bottom surface of the channel (width = 100μm). The fluid flow was driven by pressure while a DC

voltage of 2V was applied perpendicular to the axial flow. The presence of the electric field induced electroosmotic flow in the transverse direction. The staggered arrangement of the pairs of electrodes, as shown in Figure 2.10a, was reported to create a helical motion to mix the fluids. For a flow velocity of 0.5 to 1.2 mm s^{-1} , good mixing efficiency was achieved within 6 mm (10 times of the channel width).

Instead of a DC electric field, Song *et al* applied a low frequency (1Hz) square wave signal ($5V_{pp}$) across a set of embedded electrodes in a microchannel (width= $140 \mu\text{m}$, depth = $60 \mu\text{m}$), see Figure 2.10b. A mixing efficiency of 90% was achieved after a downstream distance of 1.5 mm with a flow rate of $75 \mu\text{l/hour}$. It was also reported that applying a low frequency electrical field reduced the formation of bubbles due to electrolysis, in comparison to a DC electric field.

Figure 2.10: Micromixer with integrated planar electrodes on channel floor to provide transverse (a) DC electric field (Lynn *et al.* 2008) (b) low frequency square wave electric field (Song *et al.* 2010)

Chen and Cho (2008) conducted a numerical study on a micromixer with electrodes embedded at the wall of a microchannel (see Figure 2.11). Chaotic electric signals were applied to the electrodes to stir and mix the fluids in a mixing chamber. A maximum mixing efficiency of 95% was achieved with this micromixer. However, in practice, fabrication of electrodes on the wall of microchannel is not straightforward and difficult to be implemented.

Figure 2.11: Micromixer with chaotic electric field applied at electrodes embedded in channel walls (Chen and Cho 2008)

2.7.4 Time Periodic Driving Electric Field

Utilizing time periodic electric field to enhance mixing performance has gained considerable attention lately. Lin *et al* (2004) implemented a T-form micromixer utilizing alternately switching electric field generated by a high voltage relay (see Figure 2.12a). The alternating electric field induced a wavy interface of the two fluid streams and thus increased the contact area. A maximum mixing efficiency was achieved at a certain switching frequency, depending on the magnitude of the driving electric field strength. Mixing efficiency of 97% was obtained within 1mm of the T-junction for a driving voltage of

60V/cm in 2.5s and a switching frequency of 2Hz. Meanwhile, for a higher driving voltage of 180V/cm, the maximum mixing efficiency reached 90% within 2s at a switching frequency of 8Hz.

Glasgow *et al* (2004) applied two out-of-phase square wave driving electric field to a T-channel and found improvement in mixing efficiency (see Figure 2.12b). Portions of the fluid in the inlets extended temporarily into the outlet channel and were then withdrawn back into the inlets, leading to additional stretching and folding. The flow rate and mixing efficiency at the outlet channel were reported to be unsteady if the two applied electric fields were out-of-phase by 90° because the superposition of the two electric fields at the downstream channel was not a constant.

Fu *et al* (2005) demonstrated a double T-form micromixer that utilized a periodic switching electric field. A steady electric field was applied through the first T-junction (through inlets 1 and 2) to drive the fluid flow, while a low frequency switching electric field was applied at the second T-junction (through inlets 3 and 4) to cause perturbation to the flow and thus increasing the contact area (see Figure 2.12c) in the downstream channel (width = $100\mu\text{m}$, depth = $30\mu\text{m}$). The mixing efficiency achieved was found to be dependent on the switching frequency and driving electric field strength. A maximum mixing efficiency of 97% was achieved with switching frequency of 1Hz at a downstream distance of $700\mu\text{m}$ under a driving electric field of 50V/cm.

Figure 2.12: Micro mixer utilizing (a) alternately switching electric field (Lin *et al.* 2004) (b) square wave voltages with 180° phase difference (Glasgow *et al.* 2004) (c) periodic switching (Fu *et al.* 2005) (d) sequential injection and expansion chamber (Coleman *et al.* 2006) (e) regulated AC electric field (Luo *et al.* 2008) (f) AC electric field and multiple obstacles (Yan *et al.* 2009)

Coleman *et al* (2006) demonstrated a micromixer based on symmetric sequential injection technique (Figure 2.12d). It was reported that the T-form or Y-form geometries leads to a small cross stream gradients that are difficult to reduce in order to achieve complete mixing (99%). In this cross-shaped micromixer, two mixing fluids were driven sequentially into a microchannel (width = 50 μm) by alternate switching of the voltages at the middle and side inlets. The injected fluids entered an expansion chamber downstream, resulting in a huge decrease in Pe and rapid axial diffusive mixing. Complete mixing (99% mixing efficiency) was achieved in an axial length of 2.3mm under a steady flow of 1mm s^{-1} . The final concentration of the mixed fluids can also be controlled by varying the duty cycle of the switching voltage signal. However, the expansion chamber is relatively large (width >1mm) as compared to the microchannel.

Luo *et al* (2008) performed numerical simulations on a T-form micromixer with DC electric field at one inlet and regulated AC electric field (half-wave rectification) at another inlet (see Figure 2.12e). The periodic waveform generated at the interface of the fluid streams increased the interfacial contact area and thus enhanced mixing efficiency. A maximum mixing efficiency of 92% can be achieved at a mixing distance of 0.6mm (DC electric field = 105V/cm, AC amplitude = 165V/cm, frequency = 4Hz). The DC electric field was varied to produce fluid mixtures of various concentrations. However, effective mixing can only be achieved when the width of the channel is small (channel width = 60 μm).

Yan *et al* (Yan *et al.* 2009) implemented a T-form micromixer with DC electric at one inlet and a AC electric with a DC bias at another inlet. Patterns blocks were placed along the downstream channel (width = 300 μm) to enhance the mixing efficiency. The oscillatory flow induced by the AC electric field was found to improve the mixing efficiency. Furthermore, the mixing fluids were forced to turn around bends repeatedly, thus inducing lateral velocity and yielding higher mixing efficiency as compared to a straight channel. A maximum mixing efficiency of 90% was achieved for a mixing length of 10mm

(frequency = 1Hz, DC voltage = 300V, AC voltage amplitude = 300V). The mixing length to achieve good mixing was long (33 times of the channel width) as compared to other micromixers because many blocks were required to induce significant mixing efficiency enhancement.

Applying time periodic driving electric field in combination with other mixing enhancement effects has been demonstrated by some researchers. Oddy *et al* (2001) had implemented an active micromixer that combined time periodic electric field and EKI to promote instability in the flow field. Luo *et al* (2007) had performed numerical studies on mixing enhancement by combining time periodic driving electric field and surface heterogeneity. Combining time periodic driving electric field with EKI or surface heterogeneity had shown improvement in mixing performance, as compared to that of a steady driving electric field.

2.8 Micro Mixing based on Constriction

Veenstra *et al* (1999) implemented a diffusion mixer by introducing a constriction at a certain distance after the junction of the two inlet channels as shown in Figure 2.13. An increasing pressure drop and decreasing diffusion time were observed with a decreasing constriction width. However, the mixer was found to be inefficient at higher flow rate (2 $\mu\text{l}/\text{min}$). This mixer may not be suitable for certain microfluidic devices where high throughput is required.

Figure 2.13: Diffusion micromixer with a constriction (Veenstra *et al.* 1999)

Brotherton *et al* (2008) performed numerical simulations of microchannels with single and multiple constrictions to compare the mixing performance under pressure driven flow. A mixer with constriction reduces the characteristic diffusion length of the two fluid streams and may take advantage of the entrance and exit effects to enhance mixing. A mixer with only one constriction was reported to have slightly higher mixing performance than a mixer with multiple constrictions over the same length (see Figure 2.14). The mixer relies on the shortening of transverse diffusion length through the introduction of constriction(s) in a microchannel. Therefore, the mixing region is defined to be the region with constriction. If there is only a single constriction spanning over a fixed length, the mixing region is maximized. However, if there are two or more constrictions, the mixing region is reduced because there are intermediate channels with larger width between the multiple constrictions (see Fig 2.14). Due to a reduction of mixing region over a fixed length, the mixing efficiency of the micromixer with multiple constrictions is slightly lower than the mixer with a single constriction. The mixing performance was found to increase with a decreasing constriction width. However, a smaller and longer constriction requires more power to pump the fluid and is prone to clogging if the solution contains particles or cells.

Figure 2.14: Micromixer with single and two constrictions (Brotherton *et al.* 2008)

Gan *et al* (2007) implemented an effective mixer for two dissimilar viscoelastic fluids for pressure driven flow at very low Reynolds number using an abrupt convergent and divergent geometry (see Figure 2.15). Enhanced mixing was achieved through elastically induced flow instability with negligible diffusion and inertial effect. Moreover, mixing efficiency up to 68% was achieved over a short effective mixing length at a relatively fast flow rate (12 ml/h). However, this approach requires the addition of a polymer such as polyethylene oxide (PEO) to the solution. This might not be favorable for electroosmosis as PEO might adsorb to the surface of the microchannel and alter the surface properties. In addition, a high flow rate is required to cause flow instability, implying that a rather high applied pressure is required.

Figure 2.15: (a) Micromixer utilizing viscoelastic instability through a constriction (b) Images showing viscoelastic instability mixing (Gan *et al.* 2007)

Concurrently with the work contained in this thesis, Sun and Sie (2010) developed a T-form micromixer based on sinusoidal driving pressure and a constriction attached to a diverging microchannel (see Figure 2.16a). The sinusoidal pressure at the two reservoirs was generated by the rotation of a camshaft which controlled the vertical positions of the two reservoirs and thus the pressure pulsation. The phase difference between the two pressure pulsations can be varied by adjusting the relative angle between the two cams. The formation of tendril structures and the lamination of fluid filaments (Figure 2.16b,c) were observed when the two pressure pulsations were not in phase. The phase shift affected flow inertia in the diverging channel which was critical to the onset of the hydrodynamic instability. It was also reported that a higher divergent angle (half angle) lead to a higher mixing efficiency due to the onset of flow instability in the divergent channel (see Figure 2.16d). A maximum mixing efficiency of 95.5% was achieved at 1.5mm from the T junction, with a phase shift of $\pi/2$ and a channel divergent angle of 55° .

Figure 2.16: (a) Design of micromixer based on out-of-phase pressure pulsation and constriction in a divergent channel (b) Mixing images for phase shift = $\pi/2$, half angle = 25° (c) Mixing images for phase shift = π , half angle = 25° (d) Critical Reynolds number of the onset of flow instability at various half angle of the divergent channel. (Sun and Sie 2010)

2.9 Ionic Mobility of Fluorescent Dye

Numerical flow simulations in micromixers are often conducted for the initial investigation of new micromixing scheme and optimization of mixing efficiency. The various operating parameters and

geometrical features of micromixers can be quickly adjusted without having to fabricate new devices and perform time-consuming experiments.

In micromixing experiments, various fluorescent dyes are employed for mixing visualization and evaluation. Some fluorescent dye such as Rhodamine B (Lin *et al.* 2004; Yan *et al.* 2009), fluorescein (Shin *et al.* 2007) and BODIPY 493/503 (Kim *et al.* 2009) are neutral and has negligible ionic mobility. The electromigration of such neutral fluorescent species under an applied electric field are thus negligible and are not necessary to be considered in numerical simulations. The concentration distribution of the solute can be described adequately by the conventional convection-diffusion equation (Erickson and Li 2002; Lin *et al.* 2004; Fu *et al.* 2005; Luo *et al.* 2008; Yan *et al.* 2009).

However, not all fluorescent dyes are neutral. There are also anionic (negative) and cationic (positive) dye. Positive dyes such as Rhodamine 123 (Ng *et al.* 2009) and Rhodamine 6G (Lee *et al.* 2007) dissolve in water and produce fluorescent species which are positively charged. Similarly, negative dyes such as fluorescein disodium (Gan *et al.* 2007; Lim *et al.* 2010) and BODIPY 492/515 disulfonate (Ng *et al.* 2009) dissolve in water and produce fluorescent species which are negatively charged. Few researchers (Biddiss *et al.* 2004) have included the electromigration of charged fluorescent dye in their flow simulations of electrokinetic micromixers. This electromigration effect could be significant and its omission could lead to significant error. As such, the mixing condition and concentration distribution of charged solutes or ionic species should be visualized with similarly charged fluorescent dyes, as compared to neutral dyes. However, there is no detailed research on the effect of ionic mobility of charge solutes or dye in the flow simulation of an electrokinetic micromixer and its significance on the observed flow behaviors.

The ionic mobility of a particular dye depends on the pH (Milanova *et al.* 2011), buffer concentration (Bahga *et al.* 2010) and temperature (Whang and Yeung 1992; Kim *et al.* 2006). A

conventional method to measure ionic mobility is capillary zone electrophoresis (CZE). A CZE system generally involves the injection of fluid plugs into a separation channel where the ions are separated based on their mobilities and the migration times of the ions can be determined (see Figure 2.17). The ions moving pass the detection point can be sensed with various methods, for example contactless conductivity detection (Pumera *et al.* 2002), amperometric measurement (Wang *et al.* 2007), orthogonal laser induced fluorescent detection (Whiting *et al.* 2008) and epifluorescent imaging with CCD camera (Bahga *et al.* 2010). With the conventional CZE system, measuring low mobility species may require a longer time since the injected plug will take longer to reach the detection point.

Figure 2.17: Conventional experimental setup to measure ion mobility with capillary zone electrophoresis (CZE) (Bahga *et al.* 2010)

2.10 Zeta Potential Measurement

Zeta potential is an important parameter which describes the flow rate and direction of electroosmotic flow. It is often required to be specified in the numerical simulation of electrokinetically driven

micromixers. Zeta potential depends on the concentration of buffer solution, type of channel, pH and temperature. One of the popular methods to measure zeta potential is current monitoring method.

Current monitoring was first proposed by Huang et al (1988) as a simple and convenient method to measure the electroosmotic mobility or zeta potential of a channel with uniform surface charge. The zeta potential of a microchannel is determined by measuring the system electrical current associated with the displacement flow of two fluids with a small difference in concentration. Figure 2.18 shows the original schematic diagram of the experimental setup by Huang *et al.*

Figure 2.18: Schematic experimental setup for current monitoring method to determine zeta potential. Reservoirs 1 and 2 are filled with solutions with 5% difference in concentration (Huang et al. 1988)

The polarity of the power supply is set such that the electroosmotic flow is from reservoir 1 to reservoir 2 through the capillary tube T. Capillary tube T and reservoir 2 are filled with the same electrolyte while reservoir 1 is filled with the same electrolyte but with 5% concentration difference. As the electrolyte in reservoir 1 migrates into the capillary tube by electroosmosis, it displaces an equal volume of electrolyte in the tube. As a result, the total resistance of the fluid in the capillary tube changes, and this change can be traced by recording the current over time.

When the displacement of fluid from reservoir 1 to reservoir 2 is complete, the current would become constant as the whole capillary tube is filled with fluid from reservoir 1 only. Therefore, the time interval t for the complete displacement can be estimated based on the current-time plot (see Figure 2.19). If the length of the channel L is known, the average electroosmotic velocity can then be determined easily by the equation

$$v_{eo} = \frac{L}{t} \quad (2.7)$$

The zeta potential can then be obtained by equating the Helmholtz-Smoluchowski equation with Eq. 2.7 and rearranging to give,

$$\zeta = -\frac{\mu}{\epsilon_r \epsilon_0 E} \cdot \frac{L}{t} \quad (2.8)$$

where ϵ_0 = permittivity of free space, ϵ_r = dielectric constant of the solution and E = electric field.

Figure 2.19: Typical current-time plot for current monitoring method

2.11 Direction Dependence of Displacement Time for Two-fluid

Displacement Flow

Current monitoring technique involves two fluid displacement flow, where one fluid flows and displaces another fluid with different concentration in a channel. There is experimentally observed anomaly in such flow, namely the displacement time is flow direction dependent (Gan *et al.* 2006). In other words, the time required for a low concentration solution to displace a high concentration solution is not the same as the time required for a high concentration solution to displace a low concentration solution. This displacement time difference might result in two different zeta potentials, depending on the flow direction. Hitherto, no theoretical or numerical studies have been conducted to explain this hysteretic phenomenon. This effect is not limited to zeta potential measurement. Besides current monitoring experiment, there are many other microfluidic systems involving electroosmotic flow of two or more solutions with different electric properties. The outcome of this investigation potentially creates a significant impact on the modelling of flow in a microchannel which involves multiple fluids with different conductivities or concentrations such as Field Amplified Sample Stacking (FASS) and Isotachophoresis (ITP).

Sample preconcentration and enrichment are often desirable in detection of low concentration analytes in a microfluidic device. Field amplified sample-stacking (FASS) is one of the simplest forms of preconcentration technique in microfluidic devices (Giordano *et al.* 2012). FASS has been applied to drug separation and analysis of wastewater samples (Dawod *et al.* 2010). FASS has been shown to increase the concentration of analytes and improve the sensitivity of signal detection by 100-fold to 2000-fold (Quirino and Terabe 2000; Kuban *et al.* 2001; Yang and Chien 2001).

The concept of FASS involves the setting up of an electric field across a conductivity discontinuity. Charged analytes will be stacked at the conductivity boundary when the analytes attempt

to traverse from the lower conductivity sample to a higher conductivity background electrolyte (BGE). The species move faster in the lower concentration solution because the electric field is higher in this region and thus the ions experience a higher electrophoretic velocity. As the ions cross the concentration boundary, the electric field in this high conductivity region is lower. Therefore, the ions decelerate and stack at the boundary (see Figure 2.20).

If EOF is present during FASS process, the electroosmotic flow velocity mismatch between the two solutions with different concentrations will generate an internal pressure gradient. Positive pressure gradient will be generated in the high concentration solution while negative pressure gradient will be generated in the low concentration. The presence of this internal pressure gradient ensures the continuity of fluid flow. The induced parabolic flow profile which is superimposed on the plug-like profile of EOF causes undesirable sample hydrodynamic dispersion. Various measures to suppress EOF such as coating of poly(ethylene oxide) (PEO) on microchannel have been adopted to minimize the sample dispersion (Bharadwaj and Santiago 2005). The hysteresis effect of EOF might provide an alternative way to suppress or retard EOF in FASS such that sample dispersion can be reduced to achieve better sample stacking.

Figure 2.20: Principle of Field Amplified Sample Stacking (FASS). (a) Negative sample ions are initially distributed evenly at the right side of the channel. (b) Due to the difference of electric field strength in both regions, the negative ions stack at the interface region between the two solutions.

Another well-known microfluidic application involving multiple fluids flow under an electric field is isotachopheresis (ITP). ITP is an electrophoretic separation and focusing technique used for sample preconcentration and analysis in a wide range of chemical and biological application (Garcia-Schwarz *et al.* 2011). ITP has been employed successfully to preconcentrate and separate protein (Cui *et al.* 2007) and DNA (Park *et al.* 2008). In ITP, the sample ions are sandwiched between a leading electrolyte (LE) with high mobility ions and a trailing electrolyte (TE) with low mobility ions. Under the application of the electric field on this heterogeneous solutions arrangement, the sample ions will be separated and stacked according to their electrophoretic mobilities due to the non-uniform electric field in these three regions (see Figure 2.21). Among the ions for the three regions in Figure 2.21, the mobilities of TE ions and LE ions should be the highest and lowest respectively. In many situations, electroosmotic flow occurs during the ITP process. Due to the concentration mismatch in the ITP system, EOF is non-uniform and causes sample dispersion, which has a detrimental effect on the separation resolution of this technique (Baier *et al.* 2011).

The transition zone dynamics at the boundary of sample and TE/LE has been studied and modeled numerically by Schonfeld *et al.* (2009). The model was implemented on a moving frame of reference which moves along with the concentration boundary. Thus, the model is computationally efficient and represents a simplified description of the complex transition zone dynamics in the ITP system. However, a few assumptions have been made in the model proposed. The current density boundary condition is assumed to be constant. The slip velocity boundary condition is also assumed and the flow condition does not affect the ion distribution at the EDL. As a result from these assumptions which simplify the problem significantly, hysteresis effect of electroosmotic flow cannot be predicted.

Figure 2.21: Principle of Isotachopheresis (ITP). Sample anions sandwiched between leading electrolyte (LE) and trailing electrolyte (TE) are separated and concentrated according to their mobilities due to non-uniform electric field across the channel.

These examples show that flow conditions involving multiple fluids with different conductivities or concentrations under EOF are very common in microfluidic applications. The understanding and characterization of the EOF hysteresis effect could have a significant impact on the design of microfluidic devices and the modeling of fluid flow in these devices.

2.12 Summary

Electrokinetic phenomena arise from the formation of electrical double layer through the interaction between a solid surface and the surrounding ions in an electrolyte. Electroosmotic flow is one of the electrokinetic phenomena commonly exploited in various microfluidic devices for fluid pumping. Electric field is applied across a microchannel to induce electroosmotic flow and transport analytes in these devices.

In addition to fluid transport, electrokinetic effect has been exploited to enhance micro mixing in microfluidic devices. Exploiting electrokinetic instability, introducing surface charge heterogeneity, applying time periodic driving electric field and inducing transverse electric field through planar electrode have been found to improve mixing efficiency. Constrictions have been introduced in

microchannels to enhance micromixing. However, the reported micromixers have various limitations such as restrictions on solution properties (high concentration difference between mixing streams or viscoelasticity of solutions), high voltage requirement and complex fabrication procedure (embedded metallic electrode, charged surfaces or three dimensional structures).

It is essential to develop a micromixer with a simple, compact geometry which can be fabricated easily and cheaply, and can be integrated conveniently into any commercial microfluidic system. Furthermore, a micromixer which can mix various fluids with a wide range of properties efficiently is desirable. It appears that there are no studies on combining a constriction and time periodic electric field to enhance mixing performance in a micro mixer. It would be worthwhile to investigate mixing in electrokinetic flow under time periodic electric field through a constriction.

Fluorescent dye has been used in micromixing experiments as flow markers and mixing efficiency quantifier. Fluorescent dye can be neutral, cationic (positively charged) or anionic (negatively charged). Most researchers employed neutral dyes in their experimental studies or assumed the fluorescent dyes or solute to be neutral in their numerical simulations. Little attention has been paid to the effect of charged fluorescent dye or solute in micromixing investigation. The ionic mobility of charged fluorescent dye is expected to be important in an electrokinetically driven micromixer and should be examined.

In the flow simulation of an electrokinetic micromixer, zeta potential is often required to be specified as the boundary condition. One of the common methods to measure zeta potential is by current monitoring technique, which involves one fluid displacing another fluid with different concentration. However, the two different displacement times observed depending on the flow directions imply that two different zeta potentials could be registered depending if a high concentration fluid is displacing the low concentration fluid or vice versa. It would be worthwhile to investigate this

effect because it might have potentially important impact on the accuracy of the measured zeta potential. In addition, this phenomenon has a wider implication not limited to the measurement of zeta potential as two fluid displacement flow is found in various microfluidic applications.

Chapter 3 Mixing Enhancement Mechanism

3.1 Introduction

Introducing a simple constriction at the T-junction of a T-channel under time periodic electroosmotic flow has the potential of enhancing mixing efficiency. The mixing enhancement mechanism for the proposed micromixer is described in this chapter. The mixing enhancement mechanism involves two distinct phases: formation of fluid plugs in the constriction and spreading of the plugs at the exit of the constriction. These two steps increase significantly the contact area between the two fluid streams, and thus increase significantly the mixing efficiency through the simple diffusion process. A preliminary theoretical analysis will be performed in this chapter to estimate the mixing efficiency enhancement through the increase of contact area between the two mixing fluids with the proposed micromixer.

3.2 Formation of Fluid Plugs

In a simple T-mixer, the applied voltages at the two side channels drive the fluids from the inlet reservoirs towards the main channel. The two fluid streams from the two side channels converge at the T-junctions (see Figure 3.1) and mix. If only DC electric field is applied, the position of the confluent fluid interface is fixed along the y-axis, assuming both streams have identical flow velocity.

Figure 3.1: Coordinate system at T-junction

If the applied voltages are time periodic and are not in-phase, the position of the fluid interface will not be stationary. The movement of the fluid interface is induced by the varying strength of the electric field applied at the two side channels. For this investigation, the applied electric fields are assumed to be sinusoidal with angular frequency ω , AC amplitude E_{AC} and DC offset (bias) E_{DC} . The electric fields applied at the two side channels are assumed to be out-of-phase by 180° . An expression describing the position of the confluent fluid interface at the T-junction can be derived. In this analysis, only the x component of the electric field is considered. The instantaneous electric field at the T-junction, $E(t)$ can be approximated as a sum of DC electric field and the AC electric field.

$$E(t) = E_{AC} \sin(\omega t) - E_{AC} \sin(\omega t - \pi) - Gx$$

$$E(t) = 2E_{AC} \sin(\omega t) - Gx \quad (3.1)$$

The first term in Eq. 3.1 is the difference of the two AC electric fields which are out of phase by 180° while the second term is the DC electric field. The DC electric field is approximated as a linear function of position, x , with G as the constant of proportionality. The DC electric field is in fact not

uniform at the T-junction. The approximation can be regarded as the first term of the Taylor series expansion for the electric field (Shin *et al.* 2007).

With Helmholtz-Smoluchowski equation, the governing equation for the position of the interface of the confluent stream can be written as,

$$\begin{aligned}\frac{dx}{dt} &= \frac{-\varepsilon_r \varepsilon_0 \zeta}{\mu} [2E_{AC} \sin(\omega t) - Gx] \\ \frac{dx}{dt} - \frac{\varepsilon_r \varepsilon_0 \zeta G}{\mu} x &= \frac{-2\varepsilon_r \varepsilon_0 \zeta}{\mu} E_{AC} \sin(\omega t)\end{aligned}\quad (3.2)$$

Let the initial condition be $x=0$ at $t=0$, this first order ordinary differential equation (ODE) can be solved for $x(t)$. The position of the fluid interface, $x(t)$ at steady oscillation ($t \rightarrow \infty$), may be given by

$$x(t) = \frac{-2\varepsilon_r \varepsilon_0 \zeta E_{AC} / \mu}{\sqrt{(-\varepsilon_r \varepsilon_0 \zeta G / \mu)^2 + \omega^2}} \sin\left[\omega t - \tan^{-1}\left(\frac{-\mu}{\varepsilon_r \varepsilon_0 \zeta G}\right)\right]\quad (3.3)$$

The detailed derivation of Eq. 3.3 is contained in Appendix A. Eq. 3.3 shows that the fluid interface is oscillating at a frequency ω similar to the applied AC electric field with an amplitude A given by,

$$A = \frac{-2\varepsilon_r \varepsilon_0 \zeta E_{AC} / \mu}{\sqrt{(-\varepsilon_r \varepsilon_0 \zeta G / \mu)^2 + \omega^2}}\quad (3.4)$$

The location of fluid interface x gives an indication of the composition of fluids flowing into the main channel. For example, when the fluid interface is located below the main channel, only the top fluid stream is flowing into the main channel (see Figure 3.2b). On the contrary, when the fluid interface is located above the main channel, only the bottom fluid stream is flowing into the main channel (see Figure 3.2d). This periodic movement of fluid interface allows the possible formation of alternate fluid plugs in the main channel, if certain conditions are met.

Alternate plugs of fluids can be formed when A is larger than the width of the channel which is located directly adjacent to the junction of the two side channels (see Figure 3.2). It is noted that decreasing frequency and/or increasing E_{AC} will increase A (see Eq. 3.4). Therefore, alternate fluid plugs will be observed in a channel with constriction under lower applied AC electric field as compared to a channel without constriction due to the difference of channel width immediately after the T-junction (see Figure 3.3).

Figure 3.2: Sequence for formation of trapezoidal fluid plugs in constriction. White arrows show fluid flow direction.

Figure 3.3: (a) T-channel with constriction in main channel (b) T-channel without constriction

To further understand the formation of the fluid plugs in the constriction, an expression describing the volume of the fluid plugs is derived. For a two-dimensional analysis, it is assumed that the depth of the channel is constant and only the area from the top view of the channel needs to be analyzed as the volume is directly proportional to the area. The plug of fluid can be approximated as a trapezium, assuming that the fluid interface movement is uniform at the intersection area, see Figure 3.2. The slanted sides of the trapezium are formed when the interface is in the intersection area, while the parallel sides of the trapezium are formed when the interface is above or below the intersection area.

The length of the top horizontal edge is vt_1 whereas the length of the bottom horizontal edge is vt_2 , where v is the flow velocity in the constriction, t_1 is the time for the fluid interface to move to positions $2 \rightarrow 3 \rightarrow 2$, and t_2 is the time for the fluid interface to move to positions $1 \rightarrow 2 \rightarrow 3 \rightarrow 2 \rightarrow 1$ (see Figure 3.2). The area of the trapezium is given by

$$A_{\text{trapezium}} = \frac{1}{2}(vt_1 + vt_2)w \quad (3.5)$$

where w is the width of the constriction. However it is noted that the sum of t_1 and t_2 is the period of the oscillation, T_{os} . Therefore,

$$A_{trapezium} = \frac{vwT_{os}}{2}$$

$$A_{trapezium} = \frac{vw}{2f} \quad (3.6)$$

where f is the frequency of the oscillation for the fluid interface (which is also the frequency of the applied AC electric field). This expression shows that the area of the fluid plugs formed in the constriction is inversely proportional to the frequency of the interface fluctuation.

3.3 Spreading of Fluid Plug

At the exit of the constriction, the plugs of fluid spread in a semicircular manner. Assuming that diffusion in this spreading process (in this short time frame) is insignificant, the area of the semi-circular pattern at the exit of the constriction is equal to the area of the trapezoidal plugs of fluid in the constriction. Therefore the radius of the semi-circular pattern, r can be written as

$$r = \sqrt{\frac{vw}{\pi f}} \quad (3.7)$$

However, the radius of the pattern is restricted by the downstream channel width, which is 200 μ m. r is large for low values of f . Therefore, it can be observed that for low frequencies (2Hz and 4Hz), instead of a uniform semi-circular pattern, a semi circular pattern which seems elongated in the downstream direction is formed due to the restriction of the channel wall. For simplicity, this distortion is not considered in this initial analysis.

This crescent shape layers of fluid radiating from the constriction are formed by consecutive semi-circular plugs of fluids (see Figure 3.4). This results in a significant increase of the surface to volume ratio of the two fluids and decrease of the diffusion distance of solute for complete mixing.

Figure 3.4: Sequence for spreading of fluid plugs at exit of constriction

The volume to surface ratio, R (equivalent to area to perimeter ratio in this 2D analysis) can be regarded as the ratio of the area of the crescent, which is estimated from the difference in area between the two semi-circular patterns, to the total arc length for the two semi circular pattern.

$$R = \frac{\frac{\pi}{2}(r_1^2 - r_2^2)}{\pi(r_1 + r_2)}$$

$$R = \frac{(r_1 - r_2)}{2} \quad (3.8)$$

where r_1 and r_2 are the radii of the two adjacent semi-circular pattern (see Figure 3.4b). When plug 1 is fully spread, r_1 is equal to half of the width of the main channel.

$$r_1 = \frac{W}{2} \quad (3.9)$$

r_2 can be determined by assuming that the crescent shaped area formed by the two arcs is the same as the area of the trapezoidal plug (given by Eq.3.6). Therefore, r_2 can be expressed as,

$$\frac{\pi}{2}(r_1^2 - r_2^2) = \frac{vw}{2f}$$

$$r_2 = \sqrt{\left(\frac{W}{2}\right)^2 - \frac{vw}{\pi f}} \quad (3.10)$$

Substituting Eq. 3.9 and 3.10 into Eq. 3.8 gives,

$$R = \frac{W}{4} - \frac{1}{2} \cdot \sqrt{\left(\frac{W}{2}\right)^2 - \frac{vw}{\pi f}} \quad (3.11)$$

R can also be interpreted as the characteristic length scale for the diffusion process. Eq. 3.11 shows that R decreases with increasing f . In other words, the characteristic length for the diffusion process is decreased as f increases due to the thinner layer of crescent formed.

In contrast, for confluent flow of two fluids without the formation of crescent shaped plugs, the contact area is only along the one straight edge of the stream (see Figure 3.5). For the same volume of fluid as a semi circular plug, the length of contact, L_c is

$$\begin{aligned} \frac{W}{2} \cdot L_c &= \frac{vw}{2f} \\ L_c &= \frac{vw}{fW} \end{aligned} \quad (3.12)$$

where W is the width of the main channel.

Figure 3.5: Contact length for normal diffusive mixing process

Therefore, the volume to surface ratio, R_{norm} can be calculated as

$$R_{norm} = \frac{\frac{vw}{2f}}{\frac{vw}{fW}}$$

$$R_{norm} = \frac{W}{2} \quad (3.13)$$

R_{norm} is indeed the characteristic length for diffusion process across half of the channel width.

Comparison between Eq. 3.11 and Eq. 3.13 shows that the magnitude of R is much smaller than R_{norm} .

The percentage reduction in volume to contact surface ratio is

$$\frac{R_{norm} - R}{R_{norm}} \times 100\% = 50\% + 50\% \times \sqrt{1 - \frac{4vw}{\pi W^2}} \quad (3.14)$$

Eq. 3.14 illustrates that the formation of the thin crescent shape layers reduces the characteristic length for the diffusion process by at least 50% as compared to a normal confluent flow for two fluids and thus enhances mixing significantly.

This spreading analysis so far assumes that the plugs of fluid spread uniformly and symmetrically from a trapezium pattern to a semi-circular pattern. However, the exact spreading process is in fact more complicated. Due to the slanted edge of the trapezium, the semi-circular pattern is in fact not symmetrical. The slanted portion (see Figure 3.6) of the fluid consists of two different fluids which spread separately to the edges of the channel (see Figure 3.7). This asymmetrical spreading creates an inhomogeneous mixture of two fluids and reduces the mixing efficiency.

Figure 3.6: A trapezoidal fluid plug

Figure 3.7: Sequence for unsymmetrical spreading of trapezoidal plug

The significance of this effect depends on the proportion of the slanted portions of the trapezium to the whole plug of the fluid. It is noted that the uniform part of the trapezium (the part with parallel edges) is formed when the fluid interface is above or below the constriction, while the slanted portion is formed when the fluid interface is in the intersection region (see Figure 3.2). The length of the top edge of plug 1 is given by vt_1 (see Figure 3.2f and Figure 3.6). To find t_1 , Eq. 3.3 is rewritten as

$$x(t) = A \sin(\omega t)$$

$$t = \frac{\sin^{-1}\left(\frac{x}{A}\right)}{\omega} \quad (3.15)$$

where A is the amplitude of the fluctuation as given by Eq. 3.4. The phase difference term is omitted for simplicity because it does not affect the time difference (it will be cancelled out by subtraction even if included).

It is assumed that the time for the interface to travel to positions $2 \rightarrow 3$ and $3 \rightarrow 2$ are the same. Therefore, t_1 which represents the time to travel to position $2 \rightarrow 3 \rightarrow 2$ is given by,

$$t_1 = 2 \cdot t_{2 \rightarrow 3}$$

$$t_1 = 2 \cdot \frac{\sin^{-1}\left(\frac{A}{A}\right) - \sin^{-1}\left(\frac{w}{A}\right)}{\omega}$$

$$t_1 = \frac{\pi - 2 \sin^{-1}\left(\frac{w}{A}\right)}{2\pi f} \quad (3.16)$$

where w is the width of the constriction and $\omega = 2\pi f$. The length of the bottom edge of plug 1 is given by vt_2 (see Figure 3.2f and Figure 3.6). Since the sum of t_1 and t_2 is the period of the oscillation, t_2 can be written as,

$$t_2 = \frac{1}{f} - t_1 \quad (3.17)$$

With Eq. 3.16, the ratio of the length of slanted portion to the length the trapezoidal plug, S can be written as,

$$S = \frac{v(t_2 - t_1)}{vt_2}$$

$$S = 1 - \frac{ft_1}{1 - ft_1} \quad (3.18)$$

Substituting Eq. 3.17 into Eq. 3.18 gives,

$$S = 2 - \frac{2\pi}{\pi + 2 \sin^{-1}\left(\frac{w}{A}\right)} \quad (3.19)$$

Eq. 3.19 shows that the S value increases with decreasing A . In other words, increasing f and/or decreasing E_{AC} would result in a higher S value, which means higher proportion of slanted part formed and thus less homogeneous mixing.

It is noted that for A smaller than w , the expression is not defined. This is expected because complete trapezoidal plugs are not formed when the amplitude is smaller than the width of the constriction. The limiting case is when $A = w$, $S = 1$, i.e. when the amplitude A is the same as the width of the constriction, the trapezium becomes a triangle.

Despite the simplicity of the model, it represents the mixing enhancement mechanism well. The proposed model is valid under the following conditions:

- i) The model assumes the formation of a semi-circular pattern at the exit of the constriction. The radius of the semi-circular plug, r is assumed to be smaller than one half of the main channel width, $W/2$ such that the semi-circular plug is not distorted.

$$r < \frac{W}{2} \quad (3.20)$$

Substituting r (Eq. 3.7) into Eq. 3.20 yields the lower limit for the applied frequency f for this model to be applicable.

$$f > \frac{4\nu w}{\pi W^2} \quad (3.21)$$

- ii) On the other extreme, r is assumed to be larger than the constriction width w , such that the semi-circular plug can spread into a full crescent pattern covering the entire width of the main channel.

$$r > \frac{w}{2} \quad (3.22)$$

Substituting r (Eq. 3.7) into Eq. 3.22 yields the upper limit for the applied frequency f for this model to be applicable.

$$f < \frac{4\nu}{\pi w} \quad (3.23)$$

- iii) The semi-circular plug spreading process at the exit of the constriction is assumed to be dominated by convection effect. Therefore, Peclet number is assumed to be much larger than 1.

$$\frac{\nu w}{D} > 1 \quad (3.24)$$

Short constriction length allows negligible fluid resident time for the transverse and axial diffusion of the slanted edges of the trapezoidal plug. Thus, Eq. 3.24 will be satisfied for a short constriction, and the assumption of a trapezoidal plug with defined boundaries between the two fluids within the constriction will be valid.

3.4 Summary

In summary, introducing a constriction at the T-junction under two applied out-of-phase sinusoidal AC electric fields can potentially increase mixing efficiency. Frequency f and amplitude of the interface fluctuation A are important parameters that govern the mixing efficiency. A has to be larger than the width of the constriction to allow the formation of the crescent shaped layers of fluid. Increasing f reduces the diffusion length due to the formation of a thinner layer of fluid (reduce R value) but results in a less homogeneous mixing because of the higher proportion of the asymmetrical plugs (higher S value). A balance between the two factors (R and S values) is required to achieve the highest mixing efficiency. Experimental investigations carried out to verify the mixing enhancement are reported in Chapters 4 and 5.

Chapter 4 Fabrication of Micro-mixer and Experimental Setup

4.1 Introduction

Designs and dimensions of the micro-mixer investigated are presented in this chapter. T-shaped micro-channels are commonly employed in the investigation of micro-mixing investigation. Introducing a constriction in the T-shaped micro-channel under time periodic electroosmotic flow has the potential of increasing mixing efficiency. Various micro-channel designs with constriction were proposed. All the micro-mixers proposed are two dimensional with no embedded metallic structures or electrodes and can be fabricated easily by soft lithography techniques. The fabrication of these micro-mixers and the experimental procedures for mixing efficiency evaluation are documented in this chapter.

4.2 Design of Microchannel

A straight T-channel design (Design A) is shown in Figure 4.1. Straight channels (without constriction) serve as a reference for mixing performance comparison with channels with constriction. The diameter of the reservoirs, length of the arms (side channels) and the length of the main channels are kept constant, see Table 4.1. The arm width is $100\mu\text{m}$ while the main channel width is $200\mu\text{m}$. The side channel width is set to half of the main channel width for all channels. This ensures that the average

flow velocities in the side channels are the same as the main channel for a constant volume flow rate. The reservoirs are designed to be large enough to reduce pressure driven flow caused by height difference of solution in the reservoir. The outlet reservoir is designed to be larger than the inlet reservoirs because fluids from these two inlet reservoirs will be flowing into the one outlet reservoir.

Figure 4.1: Schematic drawing of microchannel Design A (not drawn to scale)

Table 4.1: Summary of dimensions kept constant for all microchannel designs

Diameter of Inlet Reservoirs	6.0 mm
Diameter of Outlet Reservoirs	8.0 mm
Length of Side Channels	2.5 mm
Length of Main Channel	7.5 mm

For Design B1, a short constriction (40 μ m wide and 100 μ m long) is introduced right at the junction of the side channels of the T-channels, as shown in Table 4.2. Other dimensions of the channel are the same as that of the straight channel. The mixing enhancement due to the additional of the constriction will be evaluated and compared with a straight channel (Channel A). Channels B2 and B3 are designed to investigate the effect of the location of constriction on mixing efficiency. For designs B2 and B3, a constriction of similar dimension is introduced at a location 100 μ m and 300 μ m respectively, downstream from the junction of the side channels of the T-channels (see Table 4.2).

For mixer design B1, the constriction expands abruptly into a larger channel. This design introduces two sharp corners at the exit of the constriction.

Before the commencement of the experiment, the micromixer has to be pre-filled with solutions with a syringe. However, air bubbles/pockets often form at the sharp corners during the filling process, especially if the channel is fabricated with a hydrophobic material such as polydimethylsiloxane (PDMS). Continuous application of pressure on the reservoir is required to remove the air bubbles. To alleviate this problem, gradual expansion geometry can be introduced at the exit of the constriction in Design C (see Table 4.2). Mixing efficiency for this modification of design will be investigated. A gradual expansion channel with an angle of 39° (measured from the centre line) is introduced at the exit of the constriction for micro-mixer design C.

Length of constriction on mixing efficiency will be investigated. For Design D1, a gradual expansion channel is placed immediately after the T-junction without any constriction prior to it. This design represents the case of zero constriction length. For Design D2, a long constriction (40 μ m wide and 400 μ m long) is introduced right at the junction of the side channels of the T-channels (see Table 4.2).

Table 4.2: Summary of various micro-mixer designs for investigation of mixing enhancement with introduction of constriction.

Description	Micro-mixer Design		
Reference	<p>Design A</p>
Effect of Constriction Location	<p>Design B1</p>	<p>Design B2</p>	<p>Design B3</p>
Effect of Abrupt and Gradual Expansion Geometry	<p>Design B1</p>	<p>Design C</p>

<p>Effect of Constriction Length</p>	<p>Design D1</p>	<p>Design B1</p>	<p>Design D2</p>
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4.3 Fabrication of Microchannel

The proposed micro-mixers are two dimensional with simple geometries and can be fabricated easily with photolithography techniques. Photolithography is the process of transferring geometric shapes or patterns from a mask to a substrate coated with light-sensitive chemical (i.e. photo-resist).

There are two types of photoresists: positive and negative resist. For positive resist, exposure to the ultra violet (UV) light makes it soluble in a developer solution. In contrast, a negative resist after exposure to UV light becomes insoluble in a developer solution. Photolithography is a well-developed fabrication technology in the integrated circuit (IC) industry. However, in recent years, this technology has been used to fabricate micro-electro-mechanical system (MEMS) devices, such as microchannels.

4.3.1 Soft Lithography

Soft lithography is a technique based on photolithography developed for rapid prototyping of MEMS devices such as a microfluidic channel. It is called “soft” because the technique uses transparent, elastomeric polydimethylsiloxane (PDMS) with patterned relief on a surface to generate features. The PDMS microchannel can be prepared by casting PDMS against masters patterned by conventional lithographic techniques, curing and peeling the PDMS off. Negative photoresist SU8 is commonly used to create the masters patterned on a silicon wafer.

4.3.2 Fabrication of SU8 Master

A lithography mask is a stencil that transfers the pattern of a microchannel designs onto a silicon wafer or substrate coated with photoresist. Plastic mask is employed here because of its low cost and fast turn-around time. The smallest structure that can be patterned is roughly 30 μm . Alternatively, a glass mask, which is much more costly with longer turn-around time, could be used to achieve much finer

structure down to 10 μm if necessary. In this investigation, various mask designs of the microchannels are drawn using the L-edit software and the mask making is outsourced.

To create the microchannel master, SU8-50 (Microchem) is used as the photoresist to be coated onto the silicon wafers. The main process flowchart is shown in Figure 4.2.

Figure 4.2: Flowchart of SU8 Master Fabrication Process

A wafer was cleaned at the wet bench to remove impurities and contaminants on its surface. Silicon wafers were soaked in a mixture of sulphuric acid and hydrogen peroxide at 120°C for 20 minutes. The wafers were then rinsed in de-ionized (DI) water for 3 cycles. They were dried in a spin

dryer in 3 stages: 800 rpm, 2300 rpm and 2500 rpm. Dehydration baking was carried out at 120°C for 15 minutes on a hot plate (Dataplate , PMC 732 Series) to ensure that excess moistures are removed.

Negative photo-resist SU8-50 was coated on a Si wafer on a spin coater machine (CEE 100CB Spinner) to achieve a uniform thickness of 50µm. First stage spin speed was set at 500rpm for 10 seconds. 100rpm/sec acceleration was set to reach this spin speed. Second stage spin speed was set at 2500rpm for 30 seconds. 300 rpm/sec acceleration was set to reach this spin speed.

Soft baking was conducted to evaporate and harden the photo-resist film. As recommended by SU8 manufacturer, baking temperature should be slowly ramped up in two stages to achieve better coating and resist to substrate adhesion. Wafer coated with SU8 was placed on a contact hotplate. The temperature was raised to 65°C by ramping and held for 6 minutes. Temperature was raised to 95°C by ramping and held for 20 minutes. To avoid resist cracking, temperature was lowered slowly to room temperature.

Negative photoresist SU8 was polymerized under UV light exposure and rendered it insoluble in a developer solution. The plastic lithography mask was attached to a piece of glass slide. A mask aligner (Karl Suss MA6) was employed to transfer the patterns on the lithography mask to the SU8 through UV exposure. The exposure time was 50 seconds.

To enhance cross-linking in the exposed portion of the SU8 resist film, post exposure baking has to be performed. A two-step baking is recommended to prevent wafer bowing and resist cracking. Temperature was ramped to 65°C and held for 2 minute. Next, temperature was ramped to 95°C and held for 15 minutes. Temperature of the hotplate was then reduced to 25°C and the wafer was left on the hotplate for roughly 30 minutes for its slow cooling from 95°C to room temperature. Rapid cooling after post exposure will cause cracking on the pattern.

Wafer was immersed into SU8 developer solution for 5 minutes. Agitation was required to achieve better development and high aspect ratio. The region of photo-resist that did not polymerize was washed away leaving raised, patterned structures. The patterns created with SU8 were used as molding masters for replication with PDMS.

Hard baking was performed to improve the mechanical properties of the SU8 master. The master was baked at 120°C for 20 minutes. Wafer was cooled slowly to avoid cracks on the SU-8 pattern. The developed pattern was inspected under a microscope and the dimensions were measured and verified. The height of the pattern was measured using Logitech Granite Flat Master.

4.3.3 Fabrication of PDMS Microchannel

Microchannels can be fabricated by casting PDMS on the SU8 master. PDMS precursor (Sylgard 184 Silicone Elastomer, Dow Corning) and curing agent were mixed at 10:1 weight ratio. Before curing, bubbles formed during mixing of the precursor and the curing agent were evacuated by vacuum-deairing. The de-gassed PDMS was then carefully poured over the SU-8 master. PDMS was cured by baking it in an oven at 80°C for 1 hour. Once the rubbery solid PDMS was cured, it could be easily peeled off from the master. The SU8 master can be reused again to fabricate multiple copies of the PDMS channels.

By bonding the patterned PDMS channels to a glass slide, closed microchannels could be formed. Glass slides were cleaned with Isopropyl Alcohol (IPA) before bonding. Patterned PDMS was cut using a sharp pen knife into separate channels and peeled off from the wafer master.

Reservoirs were punched on the pattern PDMS with a hole puncher to allow the loading of fluids for experiments. Sealing process was performed immediately after the PDMS peeling process so as to minimize surface aging effects and to prevent dust from gathering on the PDMS surface. Both the glass

slide and PDMS were placed in the plasma machine, with the bonding surfaces facing up. Oxygen Plasma oxidation was carried out in the Reactive Ion Etching (RIE) machine with the following parameters:

Power: 100 W

Pressure: 70 mTorr

Period: 25 sec

The PDMS was then pressed onto the glass slide and baked on a hot plate for 10 minutes at 80°C.

4.4 Solution Preparation

Experiments were conducted using sodium bicarbonate (NaHCO_3) buffer solution. NaHCO_3 powder was weighted and dissolved in de-ionized water. A stock solution of 10mM NaHCO_3 was prepared. The stock solution was then diluted to 1mM for use in experiment. Employing a low concentration buffer solution would minimize Joule Heating and bubble formation when high voltage was applied to induce electroosmotic flow.

To inspect the mixing efficiency of micro-mixers, fluorescent dye was added to one of the fluid stream. Mixing efficiency could be evaluated based on the change of image intensity when the non-fluorescent fluid stream and fluorescent fluid stream came into contact and mix.

Fluorescein disodium salt ($\text{C}_{20}\text{H}_{10}\text{Na}_2\text{O}_5$) was mixed with NaHCO_3 solution to produce the fluorescent buffer solution. Filtration was carried out with a syringe filter to remove any sediment present in the solution. It was noted that adding fluorescent dye in the buffer solution increased its conductivity. The conductivity ratio between the two fluid streams might cause EKI when the voltage applied is higher than the threshold voltage. To investigate purely the effect of constriction and

sinusoidal voltage on mixing, the EKI effect has to be avoided by keeping the two mixing fluids at the same conductivity. This was achieved by controlling the concentration of the fluorescent dye to match the conductivity of the buffer solution.

The concentration of fluorescent solution prepared was 0.6 mM because it has approximately the same conductivity as 1mM NaHCO₃ solution. Therefore the addition of the fluorescent solution into the NaHCO₃ solution would not alter its conductivity significantly. The conductivity of all the solutions used in this experiment was kept constant at $90.5 \pm 1.5 \mu\text{S/cm}$.

The conductivities of all the solutions employed in the experiments were measured with a conductivity/pH meter (Radiometer Analytical, IONcheck65) to ensure consistency in results and to ensure conductivity matching between the two fluids. pH of all solutions were also measured because zeta potential and flow rate of the solution are dependent on the pH of the solutions under electroosmotic flow.

4.5 Experimental Procedure for Micro-mixing Evaluation

Buffer solution was pumped into the microchannel through the reservoirs with a syringe. Inspection was conducted under an inverted microscope (Nikon Eclipse TE2000-S) to verify that no bubbles or particles were present within the channels to obstruct fluid flow. One of the two inlet reservoirs of the microchannels was then filled with 50 μL of buffer solutions. The other inlet reservoir was then filled with 50 μL of buffer solution with fluorescent dye, see Figure 4.3. The outlet reservoir was filled with 90 μL of buffer solution. These amounts of fluid were dispensed such that the liquid level in each reservoir was the same to prevent any pressure-induced flow. A mercury lamp was employed as the illumination source to excite the fluorescent dye.

Figure 4.3: Sinusoidal electric field with amplitude E_{AC} with frequency f and DC bias E_{DC} , are applied at two inlet reservoirs of microchannel (not drawn to scale). Two waveforms are out of phase by 180°

0.5mm diameter platinum electrodes were placed in all the inlet and outlet reservoirs. DC voltage which was supplied by a high voltage amplifier (I-Bio PS300) was set to the required value and switched on. The DC voltages were set according to Table 4.3. Taking into consideration of higher electrical potential drop across the constriction, the chosen voltages ensured that the electroosmotic flow velocity which is proportional to the electric field strength remained the same for different designs. This allows the evaluation of the mixing efficiency at the main channel for all the designs at the same mean flow velocity. The frequency, amplitude and phase of the sinusoidal AC voltage were then set on a function generator (Tektronix AFG3022) and the signals were amplified with the high voltage amplifier. An oscilloscope (Hameg Instrument Combiscope) was employed to check the voltage signal supplied.

Table 4.3: Voltage applied and the corresponding electric field for different designs.

Microchannel design	Voltage applied, V	Electric field, Vcm^{-1}
A	258	258
B	270	258
C	270	258
D	300	258

Images were captured using a charged coupled device (CCD) camera (HiSense MkII) with 5 frames per second (fps). The resolution of the CCD camera is 1344 x 1024 pixels with 12 bits gray scale. 50 images were captured over 10 seconds in one experiment run and transferred to the computer through a FlowMap system hub. After the frequency was set to another value, the images were then recorded again. These experiments were repeated until all the images for the different required AC frequencies and amplitudes were recorded. From the captured images, mixing efficiency can be quantified based on the change of fluorescent dye intensity when the two streams mixed. Important parameters for the analysis of mixing mechanism such as the amplitude of fluid interface oscillation and contact area between the two fluid streams can also be obtained from the images.

A total of three repeated runs were performed to obtain a reliable set of data under the same input parameters for each channel design. The input parameters consist of combinations of six frequencies (2.1Hz, 4.1Hz, 6.1Hz, 8.1Hz, 12.1Hz and 16.1Hz) and four AC amplitudes ($1/3E_{DC}$, $2/3E_{DC}$, E_{DC} , $4/3E_{DC}$). Due to the periodic nature of the flow pattern, by having the ratio of the applied AC frequencies to the capturing frequency to be indivisible, mixing images at different phases of the whole cycle can be captured under a constant frame rate (5 frames per second). With mixing efficiency evaluated and averaged with these images, a good representation for the average mixing efficiency over the entire cycle can be obtained (refer to Appendix B for details on stroboscopic effect for capturing periodic flow

dynamics). Phase difference between the two input signals was fixed at 180° . Experiments with only DC voltage (without AC) were also performed on all designs as a reference for mixing efficiency comparison.

4.6 Image Analysis

The experimental captured images were managed by Flow Manager software. The images were then exported to bitmap (bmp) format for analysis. The intensity of the images can be correlated to the concentration of fluorescent dye. Fluorescent dye solutions with various concentrations (0.20mM, 0.15mM, 0.10mM and 0.05mM) were driven through a microchannel with electroosmotic flow. The average intensities of the images captured were evaluated with MATLAB. The concentration was normalized with the maximum concentration (0.20mM) while the pixel intensity was normalized such that the average intensity values lies between 0 and 1 (see Figure 4.4). Figure 4.4 shows clearly that the concentration of fluorescent dye and the intensity of the pixels are linearly proportional.

Figure 4.4: Linearity of fluorescent dye concentration and pixel intensity. Concentration is normalized with 0.2mM concentration. Pixel intensity is normalized such that average intensity lie between 0 and 1.

A MATLAB program is written to evaluate the mixing efficiency based on the changes of the fluorescent images when the two fluid streams mix (see Appendix C). For quantifying the degree of mixing, a method based on the standard deviation of the normalized concentration profile from that of the perfectly mixed streams was used. The mixing efficiency, σ_M is given by,

$$\sigma_M = 1 - \frac{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{c}_i - \bar{c}_\infty)^2}}{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{c}_{0i} - \bar{c}_\infty)^2}} \quad (4.1)$$

where N is the total number of points examined in the cross-stream direction, $\bar{c}_i$ is the normalized concentration at each point, $\bar{c}_{0i}$ is the normalized concentration at each point with no mixing taking place, and $\bar{c}_\infty$ is the normalized concentration in the complete mixing states.

The MATLAB program processed the images obtained from the fluorescent measurement by normalizing the intensity of each pixel. The concentration c_i at a point for a given image can be characterized as intensity value I_i , and the extreme values of concentration c_{max} and c_{min} correspond to I_{max} and I_{min} respectively (see Figure 4.5). The fluorescent intensity $\bar{I}_i$ can be normalized as

$$\bar{I}_i = \frac{I_i - I_{min}}{I_{max} - I_{min}} \quad (4.2)$$

and the mixing efficiency may be defined as,

$$\sigma_M = \left[1 - \frac{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{I}_i - \bar{I}_\infty)^2}}{\sqrt{\frac{1}{N} \sum_{i=1}^N (\bar{I}_{0i} - \bar{I}_\infty)^2}} \right] \times 100\% \quad (4.3)$$

where N is the total number of points examined in the cross-stream direction, $\bar{I}_i$ is the normalized intensity at each point, $\bar{I}_{0i}$ is the normalized intensity at each point with no mixing taking place (value 0 or 1), and $\bar{I}_\infty$ is the normalized intensity in the complete mixing states (value 0.5).

Figure 4.5: Maximum and minimum intensity in image analysis

For this investigation, mixing efficiency was evaluated over the width of the channel at a distance of $400\mu\text{m}$ to $1200\mu\text{m}$ from the T-junction (as shown in Figure 4.6). All mixing efficiencies were evaluated at various downstream locations and not the average value for the area shown. The chosen area size for mixing evaluation is based on the field-of-view provided by the microscope under a reasonable magnification. Under a larger magnification, the images captured provide clearer details of the mixing condition, at the expense of a smaller field of view for the microchannel. Therefore, the mixing efficiencies from the T-junction to a downstream distance of $400\mu\text{m}$ from the T-junction are not evaluated.

Although the interface fluctuation starts at the T-junction, the constriction only terminates after a certain downstream distance from the T-junction. Among all the micromixer designs, the longest downstream distance where the constriction terminates is $400\mu\text{m}$. It is inappropriate to measure mixing efficiency in the constriction due to the difference in width and flow velocity as compared to the main channel. Therefore, all mixing efficiencies in different micromixers are evaluated $400\mu\text{m}$ downstream of the constriction and beyond for ease of comparison.

Figure 4.6: Mixing efficiency evaluation at various downstream locations.

On the other extreme, mixing efficiency comparison was not performed at a distance too far from the T-junction because this practice defeats the purpose of the investigation on an effective micromixer which promotes good mixing in a *short distance/time*.

4.7 Summary

Various T-shaped micromixers were designed and fabricated to investigate the effect of constriction under time periodic electroosmotic flow on micro-mixing enhancement. PDMS channels were fabricated through soft lithography and sealed with glass slide to form closed channels. Two out-of-phase sinusoidal voltages were applied at the two inlet reservoirs to induce time periodic electroosmotic flow. Fluorescent dye was added to one of the two fluid streams in the experiments. Mixing efficiencies of the micromixers were evaluated by analyzing the change of image intensity when the fluid streams with and

without fluorescent dye mixed. The effects of geometrical features such as the location and length of constriction on mixing efficiency were investigated with the micromixer designs proposed. The effects of frequency and amplitude of applied AC electric field on mixing enhancement in various micromixers were also inspected. The experimental results obtained are discussed in Chapter 5.

Chapter 5 Micromixing Experimental Results

5.1 Introduction

This chapter presents the experimental results for different micromixers under various experimental conditions. The effects of constriction, frequency and amplitude of AC electric field on mixing enhancement will be discussed. The phase difference between the AC electric field was kept constant at 180° . The effect of phase difference will be investigated in the numerical studies in chapter 6. To facilitate discussion, the following symbols will be employed:

f = frequency of AC electric field

E_{DC} = DC electric field

E_{AC} = Amplitude of AC electric field

5.2 Simple T-micromixer

A simple straight T-micromixer was employed as a reference for comparison with mixing enhancement due to the introduction of constriction to the T-channel. From Figure 5.2, it can be observed that the two fluid streams were not well mixed when only a DC electric field is applied. The mixing process only occurred at the middle portion of the channel through diffusion. When two AC voltages of 2Hz with a phase difference of 180° were applied at the two inlet reservoirs, large fluctuation of fluid interface was observed at the T-junction and wavy patterns were formed at the fluid interface. For the case of $E_{AC} =$

E_{DC} and $f = 2\text{Hz}$, the large fluctuation at the T-junction formed triangular plugs of fluids which were then convected downstream (see Figure 5.1).

As the frequency was increased, the fluctuation of the fluid interface diminished gradually. Only small folding of fluid interface was observed for $f = 4\text{Hz}$ and $f = 6\text{Hz}$ while almost no folding was observed for frequencies $f \geq 8\text{Hz}$; this is likely due to the small wavy pattern blurred by the diffusion of the solutes.

Figure 5.1: Sequence of mixing images for microchannel design A ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_A = E_{DC}$, $f = 2\text{Hz}$).

Figure 5.2 Mixing condition for Design A ($E_{DC} = 258 \text{ Vcm}^{-1}$; $E_{AC} = E_{DC}$) at various frequencies.

Figure 5.3 shows the mixing efficiency along the main channel at the location of 400 μm to 1200 μm from the T-junction under various applied AC frequencies. It can be observed that the mixing efficiency at $f = 2\text{Hz}$ was the highest. This can be explained by the increase of surface area due to the large folding of the fluid interface at $f = 2\text{Hz}$. The mixing efficiency curves for other frequencies are very close to each other. It is also noted that the gradients of the curves are small and almost identical. This implies that the mixing efficiency increased very slowly with increasing downstream distance. A very long channel would be required to fully mix the two fluids in this straight T-channel.

Figure 5.3: Average Mixing Efficiency for Design A ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC}=E_{DC}$) at various frequencies. Normalized Downstream Distance = Downstream Distance / Width of Channel.

Figure 5.4 shows that the mixing efficiency (at 1.2mm downstream) achieved by applying a DC electric field was only 28%. Adding an AC electric field with $f = 2\text{Hz}$ increased the mixing efficiency to 46%. However, the folding of fluid interface was not compact due to the low frequency and that the

contact area between the two fluid streams only increased slightly. Therefore, mixing efficiency was only improved marginally at this short distance. Increasing the frequency to 4Hz induced a more compact folding of fluid interface but the amplitude of the folding was reduced significantly (see Figure 5.2). Increasing the frequency beyond 4Hz reduced the mixing efficiency gradually to 32% at $f = 16\text{Hz}$. The mixing efficiencies for all AC amplitudes were found to be almost similar.

For a channel with smaller width, the mixing efficiency would have been higher because the amplitude of the wavy pattern would be comparable to the width of the channel, as reported by Luo *et al* (2008) and Lin *et al* (2004). As the channel width increases, the wavy pattern generated by the fluctuation of the fluid interface at the T-junction could not provide sufficient folding and increase in contact area for a significant mixing enhancement.

Figure 5.4: Average mixing efficiency at 1.2 mm after T-junction of micro mixer design A at various frequencies and various E_{AC} ($E_{DC} = 258 \text{ Vcm}^{-1}$).

5.3 Introduction of Constriction

For channel design B1, a short constriction was introduced right at the T-junction of a conventional T-micromixer. From Figure 5.5, it can be observed that the two fluid streams were not well mixed when only a DC electric field was applied across microchannel Design B1. Although the constriction shortened the diffusion distance between the two fluid streams, the residence time of the fluid in the short constriction (100 μ m) was low because the flow was faster in a constriction due to the increase of the electric field strength.

When two AC voltages with a phase different of 180° were applied at the two inlet reservoirs, large fluctuation of fluid interface was observed at the T-junction. Alternate crescent-shaped layers of the two fluids were formed and radiated downstream of the constriction under the application of an AC electric field with a DC bias (see Figure 5.5). This effect increased the contact area between the two fluids significantly. The shapes and sizes of the fluid layers depended on the frequencies and amplitudes of the AC electric field.

Figure 5.5: Mixing condition for Design B1 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies.

Figure 5.6: Sequence of images at constriction for microchannel design B1 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$, $f = 2\text{Hz}$).

Figure 5.6 illustrates the sequence of images of the flow at the constriction. At $t=0$, the electric field for the bottom inlet was stronger and the fluid from the bottom flowed towards the top (the interface moves upwards). This allowed only the fluid from the bottom to flow into the constriction. At $t=0.4$, the electric field for the top inlet was stronger and the fluid from the top flowed towards the bottom (the interface moved downwards). This allowed only the fluid from the top to flow into the constriction. These time periodic actions formed alternate plugs of fluids in the constriction. The plugs of fluids then broaden and radiated out from the constriction in a semi-circular manner at the sudden expansion channel. The plugs became thin crescent-shaped layers of fluids because they occupied a channel with a larger width when exiting the constriction to maintain flow continuity. The spreading and thinning effect increased the contact area between the two fluids and reduced the diffusion length, thus enhanced the mixing efficiency significantly.

5.4 Effect of AC Electric Field Amplitude and Frequency

From Figure 5.7, it can be observed that the mixing efficiencies at $f = 2\text{Hz}$ was slightly lower than the mixing efficiency achieved by just applying a DC electric field at a distance of $400\mu\text{m}$ downstream from the T-junction (corresponding to the normalized downstream distance = 0 in the figure). This is due to the formation of large crescent shaped layers of fluids, which consisted of a large portion of unmixed fluids. However, the transverse concentration gradient of the crescent layers of fluids induced diffusion of solute. Therefore, the mixing efficiency increased as the layers of fluid flowed downstream. However, Figure 5.8 indicates that the standard deviation for the average mixing efficiency at $f = 2\text{Hz}$ was very large as compared to other frequencies. This was caused by the uneven distribution of solute due to the formation of the large plugs of fluid.

Figure 5.7: Average Mixing Efficiency for Design B1 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies. Normalized Downstream Distance = Distance / Width of Channel

Figure 5.8: Average mixing efficiency at 1.2 mm after T-junction of micro mixer design B1 at various frequencies and E_{AC} ($E_{DC} = 258 \text{ Vcm}^{-1}$)

As the frequency increased to 4Hz, thinner and more compact crescent shaped layers of fluids were observed. The thin layers of fluid and the large contact area developed enhance mixing through diffusion. Therefore, the mixing efficiency for $f=4\text{Hz} \sim 6\text{Hz}$ increased rapidly with increasing downstream distance (see Figure 5.7). A maximum mixing efficiency of 84% was achieved at $f = 4 \sim 6\text{Hz}$ and $E_{AC} = 4/3 E_{DC}$ (see Figure 5.8). This frequency range is the optimum frequency range for maximum mixing efficiency. As frequency increased beyond 6Hz, the mixing efficiency reduced gradually. Figure 5.5 shows that the crescents formed at high frequencies did not cover the entire width of the channel. This caused the formation of unmixed fluids near the walls of the channel, resulting in an inhomogeneous mixture of the solutions.

The homogeneity of the solutions can be inspected by plotting the concentration profile across the width of the channel at various frequencies. Figure 5.9 shows that the concentration was quite

uniform at $f=4\text{Hz}$ and low concentration gradient across the channel was observed. However, as the frequency increased, the concentration gradient across the width of the channel increased significantly. This deviation of the concentration profile from the fully mixed state (normalized concentration = 0.5) caused the mixing efficiency to drop significantly as the applied AC frequency increased.

Figure 5.9: Normalized concentration distribution across width of micro mixer design B1 (at 1.2mm after T-junction) with applied AC amplitude $E_{AC} = 4/3 E_{DC}$. Cross stream distance is normalized with width of channel (200 μm)

Figure 5.8 clearly shows that the mixing efficiency increased with increasing applied AC amplitude at all frequencies. Figure 5.10 shows the cross-stream concentration profile for various AC amplitudes at a constant frequency. From Figure 5.10, it can be clearly observed that the concentration gradient across the channel width increased with decreasing AC amplitude. This indicates that at low AC amplitude, the fluids near the two edges of the walls were not well-mixed. The drop in mixing efficiency

with increasing frequency and/or decreasing AC amplitude have been predicted by the preliminary theoretical analysis in Chapter 3.

Figure 5.10: Normalized concentration distribution across width of micro mixer design B1 (at 1.2mm after T-junction) with applied AC frequency 6.1Hz. Cross stream distance is normalized with width of channel (200 μ m).

5.5 Effect of Constriction Location on Mixing Efficiency

Experiments in Section 5.3 have shown that mixing efficiency was enhanced significantly with the introduction of constriction immediately after the T-junction. In this section, the experimental results for T-micromixer with a constriction (with similar length and width) located at 100 μ m or 300 μ m (see Figure 5.11) further downstream from the T-junction is examined and discussed.

Figure 5.11: Designs of T-micromixers with constriction introduced at different locations.

When two AC voltages of 2Hz with a phase different of 180° were applied at the two inlet reservoirs, large fluctuation of fluid interface (see Figure 5.12) was observed at the T-junction of Design B2 and B3. The downstream folding pattern did not differ significantly from a straight channel (design A) under the same parametric values although the patterns appeared to be fuzzier due to the diffusion in the constriction (see Figure 5.13).

Figure 5.14 shows that the maximum mixing efficiencies of design B3 only improved slightly (up to a maximum of 55%) as compared to a simple straight T-mixer (49%) under the same applied AC amplitude and frequency. The slight increase of mixing efficiency is expected due to the shortening of diffusion distance in the constriction. Figure 5.15 illustrates the mixing efficiency for mixer design B under various applied AC amplitudes and frequencies. Similar to a straight channel (design A), the maximum mixing efficiency was achieved with $f=2.1\text{Hz}$ and $E_{AC}=4/3 E_{DC}$. The formation of a large wavy pattern at $f = 2\text{Hz}$ enhanced mixing efficiency. The mixing efficiencies for $f \geq 6\text{Hz}$ did not differ significantly, because the small wavy pattern at the interface was blurred by diffusion.

Figure 5.12: Sequence of images at the constriction for microchannel design B3 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$, $f = 2\text{Hz}$).

Figure 5.13: Mixing condition for Design B3 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies.

Figure 5.14: Average Mixing Efficiency for Design B3 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies. Normalized Downstream Distance = Distance / Width of Channel

Figure 5.15: Average mixing efficiency at 1.2 mm after the T-junction of micro mixer design B3 at various frequencies for various E_{AC} ($E_{DC} = 258 \text{ Vcm}^{-1}$)

Figure 5.16 shows the mixing efficiency evaluated at 1.2mm downstream from the T-junction for the three designs at different AC amplitudes and frequencies. For small AC amplitude ($E_{AC}=1/3E_{DC}$), the mixing efficiency for each design was almost similar although the mixing efficiency for Design B1 was slightly higher at $f=2.1\text{Hz}$ and 4.1Hz . For higher AC amplitude, the mixing efficiency for design B1 was significantly higher than the other two designs for all frequencies.

The maximum mixing efficiency for design B1 were observed at $f=4.1\text{Hz}$. Mixing efficiencies at $f=4.1\text{Hz}$ for the three designs are plotted against the distance of the constriction from the T-junction in Figure 5.17. Distance of constriction at $0\mu\text{m}$, $100\mu\text{m}$ and $300\mu\text{m}$ correspond to mixer designs B1, B2 and B3 respectively. Mixing efficiency for the case of $E_{AC}=4/3E_{DC}$ decreased from 83% to 55% when the constriction was shifted $100\mu\text{m}$ downstream from the T-junction. Similarly, mixing efficiency decreased from 79% to 51% for $E_{AC}=E_{DC}$ and from 70% to 45% for $E_{AC}=2/3E_{DC}$. Shifting the constriction further away to $300\mu\text{m}$ caused the mixing efficiency to decrease even lower to 40~43%.

Figure 5.16: Mixing efficiency at 1.2mm downstream from T-junction for micromixer design B1, B2 and B3 at various AC frequencies. Applied DC electric field

$E_{DC} = 258 \text{ Vcm}^{-1}$ and AC electric field (a) $E_{AC} = 1/3 E_{DC}$ (b) $E_{AC} = 2/3 E_{DC}$ (c) $E_{AC} = E_{DC}$ (d) $E_{AC} = 4/3 E_{DC}$.

Figure 5.17: Mixing efficiency (for f , AC frequency = 4Hz, evaluated at 1.2mm downstream from T-junction) for T-mixers with constriction located at various distance from T-junction for different AC amplitude (E_{AC}). $E_{DC}=258\text{Vcm}^{-1}$.

Mixing enhancement with mixer design B1 relied on the formation of alternate plugs of fluid in the constriction. The plugs of fluids would then spread into thin crescent shaped layers at the sudden expansion geometry. The alternate crescent layers of fluid shortened the characteristic length for diffusion and increased the mixing efficient (see Figure 5.18a). The alternate plugs of fluid would only be formed in the constriction if the channel immediately after the T-junction was smaller than the oscillation amplitude of the two-fluid interface. The constriction for mixer design B1 was fully occupied by only one of the fluids at a time, forming a full plug. However, for mixer designs B2 and B3, the channel width immediately after the T-junction was larger than the fluid oscillation amplitude and wavy patterns were formed (see Figure 5.19). The constrictions which were further downstream could not be

completely filled with one fluid at a time (in the width direction) and a complete plug was not formed in the constriction (see Figure 5.18b and c).

(a) Design B1

(b) Design B2

(c) Design B3

Figure 5.18: Comparison of mixing condition for design A1, A2 and A3 ($f=4\text{Hz}$, $E_{AC}=E_{DC}$, $E_{DC}=258\text{Vcm}^{-1}$)

For a complete plug at the exit of the constriction, its spreading is symmetrical resulting in a significant increase in the contact area for the two fluids. The characteristic length for diffusion is then reduced and mixing is enhanced. However, for a plug which is incompletely filled in the width direction, its spreading is asymmetrical and the two fluids are separated to the opposite edges of the channel respectively (see Figure 5.20). Mixing efficiency is thus significantly reduced.

The mixing enhancement mechanics is compared with the Taylor hydrodynamic dispersion effect (Taylor 1954). Taylor's dispersion refers to an apparent increase in diffusivity of a species due to a shear flow as compared to a plug flow. This is because a parabolic flow profile generates both radial and axial concentration gradient. However, in the proposed micromixer, the flow has a typical plug-like flow profile of electroosmotic flow, and differs from the parabolic flow profile in a shear flow or pressure driven flow. The shape evolution of the crescent produced by the micromixer through channel expansion is different from a typical crescent created by Taylor dispersion. The latter (parabolic flow with Taylor dispersion) will elongate indefinitely as the crescent is transported downstream because the flow velocity at the middle of the channel is higher than the channel wall/edge. However, the plug like

flow in the proposed micromixer will not elongate as the crescent is convected downstream because the velocity throughout the cross section is the same.

Figure 5.19: Fluid interface oscillation at T-junction for mixer design B2 & B3. White arrows indicate fluid flow directions. A = amplitude of fluid interface oscillation.

Figure 5.20: Asymmetrical spreading for incomplete fluid plug at the exit of constriction

5.6 Effect of gradual expansion on mixing efficiency

Micromixer design C is similar to design B1, except that the sudden expansion at the exit of the constriction is modified to a gradual expansion (Figure 5.21). The expansion process which is required to increase the contact area for the two fluids still occur even if the channel expands gradually instead of abruptly (see Figure 5.22). Figure 5.23 shows that mixing efficiencies for the two designs are almost similar. Therefore, the expansion angle of micromixer could be modified if required without comprising the mixing efficiency.

(a) Design B1

(b) Design C

Figure 5.21: Comparison between abrupt expansion and gradual expansion at exit of constriction.

(a) Design B1

(b) Design C

Figure 5.22: Comparison of mixing condition for Design B1 and C. ($f=4\text{Hz}$; $E_{AC}=E_{DC}$; $E_{DC}=258\text{Vcm}^{-1}$)

Figure 5.23: Mixing efficiency at 1.2mm downstream from T-junction for micromixer design B1 and C at various AC frequency. Applied DC electric field $E_{DC} = 258 \text{ Vcm}^{-1}$ and AC electric field (a) $E_{AC} = 1/3 E_{DC}$ (b) $E_{AC} = 2/3 E_{DC}$ (c) $E_{AC} = E_{DC}$ (d) $E_{AC} = 4/3 E_{DC}$.

5.7 Effect of constriction length on mixing efficiency

The effect of constriction length on mixing efficiency is investigated with designs D1 and D2 (see Figure 5.24). For Design D1, a gradual expansion channel is placed immediately after the T-junction without any constriction prior to it. Experimental results in Section 4.5 show that the expansion angle does not affect the mixing efficiency significantly. Therefore Design D1 can be approximated as a mixer with zero constriction length and compared with the other two designs.

Figure 5.24: Designs of T-micromixers with various constriction lengths.

Microchannel design D2 has a longer constriction at the T-junction as compared to microchannel design C. Figure 5.25 shows the formation of alternate crescent-shaped fluid layers for Design D2. Figure 5.26 shows the sequence of images at the T-junction and the formation of the crescent-shaped fluid layers.

Figure 5.27 indicates that by applying an AC electric field with a DC bias driving electric field increases the mixing efficiency for all frequencies as compared to just applying a DC electric field. Mixing efficiencies were not high (ranged from 20% to 40%) at the exit of the constriction for all applied frequencies. However, with the formation of crescent-shaped patterns, the mixing efficiency increased rapidly after a short downstream distance after the constriction. Figure 5.28 shows that the highest mixing efficiency of 92% was achieved with $f = 6\text{ Hz}$ and $E_{DC} = 4/3 E_{AC}$. Mixing efficiency reduced gradually

beyond $f = 6\text{Hz}$. The characteristic trend of mixing efficiency under the variation of AC frequency and amplitude is very similar for the three designs (B1, D1, and D2) because the mixing mechanisms are basically identical. However, the exact values of mixing efficiencies for these three mixers are different due to the difference in their constriction length.

Figure 5.29 shows that Design D2 which has a longer constriction yielded higher mixing efficiencies under various AC amplitudes and frequencies. The plugs formed in the constriction due to the fluid interface oscillation can be approximated as a trapezium (see Chapter 3). The constriction plays the role of smoothing the slanted edges (by facilitating diffusion process) of the trapezoidal plugs formed. Slanted edges of the trapezoidal plugs lead to asymmetrical spreading during the expansion process and thus a lower mixing efficiency. The smoothing of slanted edges will cause the plugs to spread into more symmetrical crescent-shaped pattern (see Figure 5.30) at the expansion geometry, resulting in a more homogeneous mixture of fluids. Comparing Figure 5.9 (100 μm constriction length) and Figure 5.31 (300 μm constriction length), it can be observed that the cross-stream concentration gradient was much lower in the micromixer with longer constriction. The mixing efficiency is maximized when the concentration gradient of the fluid plug is reduced in the constriction before the spreading process.

Figure 5.25: Mixing condition for Design D2 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies.

Figure 5.26: Sequence of images at the constriction for microchannel design D2 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$, $f = 2\text{Hz}$).

Figure 5.27: Average Mixing Efficiency for Design D2 ($E_{DC} = 258 \text{ Vcm}^{-1}$, $E_{AC} = E_{DC}$) at various frequencies. Normalized Downstream Distance = Distance / Width of Channel

Figure 5.28: Average mixing efficiency at 1.2 mm after T-junction of micro mixer design D2 at various frequencies and E_{AC} ($E_{DC}=258 \text{ Vcm}^{-1}$)

The effect of smoothing is more prominent at higher frequency because of the ratio of slanted edges to the straight edges of the trapezoidal plug increases with increasing AC frequency (see Eq. 3.19 of Chapter 3). Design D1 yielded the lowest mixing efficiency especially at high frequencies due to the absence of constriction. With a longer constriction, Design D2 was able to maintain higher mixing efficiencies even at higher frequencies (see Figure 5.29). Figure 5.32 shows the relationship between mixing efficiency and constriction length at a constant frequency ($f=16\text{Hz}$). Mixing efficiencies clearly increased with increasing constriction length for all AC amplitudes.

Figure 5.29: Mixing efficiency at 1.2mm downstream from T-junction for micromixer design A1, A5 and A6 at various AC frequency. Applied DC electric field $E_{DC} = 258 \text{ Vcm}^{-1}$ and AC electric field (a) $E_{AC} = 1/3 E_{DC}$ (b) $E_{AC} = 2/3 E_{DC}$. (c) $E_{AC} = E_{DC}$ (d) $E_{AC} = 4/3 E_{DC}$.

Figure 5.30: Mixing condition for constriction length = (a) 300 μm , (b) 100 μm and (c) 0 μm , showing degree of symmetry for crescent-shaped pattern formed. ($f=8\text{Hz}$, $E_{AC}=E_{DC}$, $E_{DC}=258\text{Vcm}^{-1}$)

Figure 5.31: Normalized concentration distribution across width of micro mixer design D2 (at 1.2mm after T-junction) with applied AC amplitude $E_{AC} = 4/3 E_{DC}$. Cross stream distance is normalized with width of channel (200 μm)

Figure 5.32: Mixing efficiency (for AC frequency = 16Hz, evaluated at 1.2mm downstream from T-junction) for T-mixers with various constriction length for different E_{AC} ($E_{DC}=258\text{Vcm}^{-1}$)

5.8 Summary

A simple straight T-mixer under a driving DC electric field yields low mixing efficiency. When two out-of-phase sinusoidal electric fields with a DC bias are applied at the inlets of the T-mixer, fluid interface fluctuation is observed at the T-junction. Introducing a constriction at the T-junction allows the formation of alternate crescent-shaped layers of fluid downstream. This effect increases the contact area of the two fluids significantly and enhances mixing efficiency.

The optimum frequency for maximum mixing efficiency is 4~6Hz for these micromixers. Mixing efficiency is observed to increase with higher applied AC amplitude. Mixing efficiency is also shown to be dependent on the location and length of the constriction. Mixing efficiency reduces with increasing downstream distance of the constriction away from the T-junction while increases with increasing constriction length.

Chapter 6 Numerical Simulation of Micromixing under Electroosmotic Flow

6.1 Introduction

The theoretical model proposed in Chapter 3, although elucidated well the mixing mechanism, lacks sufficient accuracy for a good representation of the flow field, and thus not suitable for the determination of the operation conditions for optimum mixing. Thus, a numerical model was developed and presented in the chapter. Numerical simulations for flow in the micromixer were performed with finite element method (FEM) software: COMSOL Multiphysics. The importance of including ionic mobility of fluorescent dye in micromixing simulation under electroosmosis is illustrated here. The mixing enhancement mechanism is analyzed with the numerical simulation results.

6.2 Governing Equations

The simulation domain based on micromixer Design B1 (see Chapter 4) is shown in Figure 6.1. In the simulation, only one fifth of the length of the microchannel was simulated to save computation resources without sacrificing simulation accuracy.

Figure 6.1: Simulation domain and dimensions

Electric field is applied across the two inlets and outlet to generate electroosmotic flow. The governing equation to describe the electric potential distribution in a neutral medium is given by the Laplace equation:

$$\nabla \cdot \nabla \phi = 0 \quad (6.1)$$

where ϕ = electric potential.

Charge accumulation at the surface of the microchannel produces the electrical double layer, which has a non-zero charge density. The applied electric field exerts force on this thin charged layer and induces fluid movement. After obtaining the electric potential distribution, the flow field can be solved with the continuity equation and the Navier-Stoke equation for an incompressible Newtonian fluid:

$$\nabla \cdot \mathbf{u} = 0 \quad (6.2)$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{F}_e \quad (6.3)$$

where $\mathbf{u}$ = fluid velocity, ρ = fluid density, p = pressure, μ = fluid dynamic viscosity and $\mathbf{F}_e$ = external force exerted by the electric field on the fluid. Three models are typically employed to couple the electric field applied to the fluid movement, namely: slip velocity model, Poisson-Boltzmann (PB) model and Nernst-Planck (NP) model. PB and NP models require solving additional equations which describe the positive and negative ion distributions in the solution. These two models require very fine mesh at the boundary of the wall (in the vicinity of the EDL layer) to resolve the steep changes of wall static potential and ion distribution.

A simpler model, the slip velocity model ignores the velocity variation near the wall and assumes that the flow is induced by a moving wall with a slip velocity, given by:

$$v_{eo} = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} E \quad (6.4)$$

where ε_0 = permittivity of free space, ε_r = relative permittivity, ζ = zeta potential and E = applied electric field. In the slip velocity model, the external force $\mathbf{F}_e$ in Eq. 6.3 is set to 0 since there is no net charge in the bulk of the solution. Despite its simplicity, the slip velocity model can describe well the bulk fluid flow under time periodic electroosmotic accurately (Marcos *et al.* 2004; Yan *et al.* 2009). Therefore, the slip velocity model is adopted in this numerical analysis. Slip velocity boundary condition is valid under the following conditions:

- i) Channel dimension is large with respect to the Debye layer thickness. Therefore, the overlapping of EDL does not occur.
- ii) The flow is predominantly viscous with $Re < 1$.

Ionic dyes are widely employed in the various studies of micromixers due to its high solubility in aqueous buffer solutions. In most of the numerical simulations of flows in micromixers, the dye concentration distribution is often described by the convection-diffusion equation:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = -\mathbf{u} \cdot \nabla c \quad (6.5)$$

where c = concentration of dye and D = diffusion coefficient of dye. The dye is often assumed to be convected by the fluid flow (Lin *et al.* 2004; Fu *et al.* 2005; Yan *et al.* 2009) and unaffected by electromigration due to the applied electric field. This is only true if the dissolution of the dye will not result in charged species. However, for disodium fluorescein dye, the fluorescein ion (with -2 ion charge number) is charged and not electrically neutral. For charged ion species, the Nernst-Planck equation should be employed:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot [-D \nabla c_i - u_{mi} c_i \nabla \phi] = -\mathbf{u} \cdot \nabla c_i \quad (6.6)$$

where c_i = concentration of ionic dye and u_{mi} = ionic mobility. The third term on the left side of Eq. 6.6, which is often neglected, describes the electromigration effect on the charged dye concentration distribution. Eq. 6.6 reduces to Eq. 6.5 if electromigration is neglected. The effect of electromigration of charged dye in flow simulation of micromixers will be considered explicitly here as it was discovered that its omission will lead to significant error in the predictions of flow behavior and mixing efficiency.

The electromigration of fluorescein ion consists of two components: migration due to the applied electric field across the channel to drive the flow and due to electric potential from the charged channel wall. Only the electromigration due to the applied electric field was included in the model. The electromigration component due to the charged wall has been neglected because it diminishes after a small distance away (in the order of tenths of nanometer) from the wall due to the shielding effect of electrical double layer. The error induced by this assumption will be discussed in Section 6.10. Two important parameters in the simulation, namely the zeta potential and the mobility of fluorescent dye are measured in Sections 6.3 and 6.4.

The other ions in the mixing streams, namely sodium ion (Na^+) and bicarbonate ion (HCO_3^-), are the dominant ions which are present in much higher concentrations and govern the electroosmotic flow velocity and direction. However, electromigrative movements of these buffer ions are not explicitly considered in the numerical simulation because they have been accounted by the slip-velocity model. The accumulation of cations (Na^+ ions) and the repulsion of anions (HCO_3^- ions) from a negatively charged wall, form the electrical double layer (EDL). Under an applied electric field, these cations and anions move under electromigration effect, and thus induce electroosmotic flow of the bulk fluids. The slip velocity model prescribes a slip velocity (Eq. 6.4) at the wall to account for the fluid movement induced by these buffer ions movements in the EDL.

6.3 Measurement of Zeta Potential

Zeta potential is an important parameter which governs the flow rate and direction of electroosmotic flow. It is dependent on the microchannel material, temperature, pH, concentration and type of solutions. Although values of the zeta potentials for various solutions are reported in the literature, it is worthwhile to measure zeta potential experimentally with the exact parametric condition of the micromixing investigation. This will ensure that the zeta potential obtained is as accurate as possible.

A straight PDMS microchannel (length=5.5cm, width=40 μm and height=50 μm) was fabricated with the soft lithography process mentioned in Chapter 4. It was then bonded to a glass slide through oxygen plasma treatment. Two reservoirs (diameter of 2cm and height of 0.5cm) were fabricated from PDMS and attached to the inlet and outlet of the microchannel.

A high voltage power supply (CZE1000R, Spellman) was employed to provide the electric field for inducing electroosmotic flow. The current was monitored by a picoammeter (Keithley 6485) which

was connected in series to the microchannel (see Figure 6.2). A Labview program was written to control the two devices and to obtain the voltage and current data through a data acquisition card (PCI-6052E, National Instrument).

The buffer solution employed in micromixing studies is 1mM NaHCO_3 solution (pH=7.2). The microchannel was first flushed for 5 minutes with 1mM NaHCO_3 solution with electroosmotic flow. A steady current was obtained because the solution concentration along the channel was uniform. Reservoir 1 was then filled with 0.95mM NaHCO_3 solution while reservoir 2 was filled with 1mM NaHCO_3 solution. A voltage of 550V was applied to induce a displacement flow. The lower concentration solution from reservoir 1 displaced the 1mM buffer solution in the microchannel. The experiment was stopped when a steady value of current was achieved and the displacement time was determined from the current-time plot (see Figure 2.19). The experiment was performed up to 5 times to ensure consistency of the measured displacement time. Zeta potential of the microchannel was calculated with the slip velocity equation (Eq. 6.4).

Figure 6.2: Experimental setup for current monitoring method with picoammeter.

Fluorescent dye was added to one of the fluid streams in the micromixing studies to examine the mixing efficiency between the two streams. It has been reported that caged fluorescent might change the zeta potential of glass and polymer microchannels. Caged fluorescent dye is a special type of fluorescent dye which is made non-fluorescent through the binding of certain chemical groups. These “caging” groups can be removed by exposing the dye to UV light, thus restoring the dye to its uncaged (and fluorescent) state. Although the dye (disodium fluorescein) that was employed in the micromixing studies in Chapter 5 is not a caged fluorescent dye, it is nevertheless worthwhile to examine the effect of the dye on zeta potential of the microchannel. Therefore, the displacement time and zeta potential for the buffer solution with fluorescent dye was also measured with the current monitoring method.

The zeta potential of the PDMS/glass microchannel in the buffer solution was found to be -59.8mV (with a standard deviation of 2.2mV) based on five experimental runs. The zeta potential for 1mM NaHCO_3 buffer solution mixed with 0.06mM of disodium fluorescein dye was measured to be -60.9mV (with a standard deviation of 4.0mV). To further verify the effect of ionic dye on flow rate and zeta potential, the same buffer solution was mixed with another commonly used fluorescent dye, i.e. 0.03mM Rhodamine B. Its zeta potential was measured to be -57.5mV (with a standard deviation of 2.9mV). With and without the addition of the dyes, the differences in zeta potentials measured were well within one standard deviation. Thus, the addition of a small amount of fluorescent dyes such as disodium fluorescein and Rhodamine B to the buffer solution does not modified the zeta potential significantly. Therefore, a constant zeta potential was assumed throughout the whole channel.

To ensure that the addition of fluorescent dye did not alter other fluid properties, the displacement times for fluorescent and non-fluorescent buffer solution were measured through current monitoring method. Although direct measurements of viscosity were not performed in this study, the measurement of displacement time through current monitoring method is a reasonable and more

holistic indicator for any changes in flow rate and other fluid properties which affect electroosmotic flow behavior (for example viscosity and permittivity). Displacement time, t can be obtained by re-arranging the Smoluchowski slip velocity equation (Eq. 6.4).

$$t = -\frac{\mu L}{\varepsilon_r \varepsilon_0 \zeta E} \quad (6.7)$$

If any other flow properties of the fluid were modified when the fluorescent dye was added, the displacement time would have differed from the non-fluorescent buffer solution. At an applied voltage of 550V over a PDMS-glass microchannel of 5.5cm, the displacement time for non-fluorescent buffer solution was measured to be 118s (with a standard deviation of 4.6s). The displacement times for buffer solutions with disodium fluorescein and Rhodamine B were 109s (with a standard deviation of 7.0s) and 120s (with a standard deviation of 5.9s) respectively. This experiment confirmed that the electroosmotic flow displacement time for the fluorescent buffer solution did not differ significantly from the non-fluorescent buffer solution. Therefore, it can be inferred that the fluid properties did not change significantly due to the addition of a small amount of fluorescent dye such as disodium fluorescein and Rhodamine B.

6.4 Measurement of Fluorescent Ion Mobility

The configuration of the micromixer proposed in Chapter 5 is rather similar to the conventional apparatus for measuring ion mobility with capillary zone electrophoresis (CZE). The two out-of-phase sinusoidal AC electric fields induce the formation of alternate fluid crescents through the constriction at the T-junction. The fluorescent property of fluorescein ion allows the direct observation of the movement of these fluid crescents through CCD camera. Based on this concept, a simple method was proposed to estimate the mobility value of fluorescent ion species.

The experimental setup is the same as the micromixing enhancement investigation described in Chapter 4. Consecutive fluorescent images for the case of AC frequency $f=2.1\text{Hz}$ at the exit of the constriction were captured with CCD camera. The ionic mobility could be determined by inspecting the velocity of the moving crescent-shaped pattern. These images were post-processed with MATLAB and the intensity profiles along the centreline of the channel for two consecutive images were obtained. According to Nyquist criterion, the capturing frequency has to be more than two times the characteristic frequencies of the flow pattern to avoid aliasing. In this case, the Nyquist criterion was fulfilled because the capturing frequency was 5Hz while the frequency of the flow pattern inspected was 2.1Hz. By inspecting the shift of peaks for the two consecutive intensity profiles, the velocity of the moving crescent pattern could be obtained (see Figure 6.3). This shift distance divided by the time interval gave the velocity of the moving crescent, v_c .

The velocity of the moving crescent is the result of two transport effects: fluid electroosmotic flow and electromigration of fluorescent dye. After deducting the contribution from electroosmotic velocity v_{eo} , the velocity component due to electromigration v_m can be determined:

$$v_m = v_c - v_{eo} \quad (6.8)$$

The electromigration velocity v_m is related to ionic mobility u_{mi} :

$$v_m = -u_{mi} |\nabla \phi| \quad (6.9)$$

Substituting the slip velocity equation (Eq. 6.4) and Eq. 6.9 into Eq. 6.8, and rearranging, an expression for ionic mobility is obtained:

$$u_{mi} = -\frac{1}{|\nabla \phi|} \left(v_c - \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} |\nabla \phi| \right) \quad (6.10)$$

The negative gradient of applied electric potential ϕ is the applied electric field. The electric field is expected to be non-uniform at the vicinity of the constriction due to the abrupt change in

geometry. Numerical simulation shows that the electric field is uniform after approximately 200 μm downstream from the constriction. Therefore, only intensity profiles more than 200 μm downstream from the constriction were analyzed because the electric field was uniform and could be easily determined from numerical simulation.

Figure 6.3 Experimental images at T-junction for AC frequency $f=2.1\text{Hz}$, AC amplitude $E_{AC}=4/3E_{DC}$ at (a) $t=0$ and (b) $t=0.2\text{s}$. $E_{DC} = 258 \text{ Vcm}^{-1}$. (c) Intensity profile along centerline of microchannel in two consecutive images. Velocity of moving crescent can be estimated from shift in intensity peak.

Zeta potential for the solution can be determined with current monitoring method (see Section 6.3). Therefore the electroosmotic flow velocity v_{eo} can be calculated with the Smoluchowski slip velocity equation. Since the electroosmotic flow profile is plug-like, the flow velocity is identical regardless of the location across the width or depth of the channel.

To verify the accuracy of the proposed method, the mobilities of disodium fluorescein in various concentrations of HEPES buffer solution were measured and compared with the theoretical and experimental value obtained by Bahga *et al* (2010). The pH for the HEPES buffer solution was adjusted with 50mM sodium hydroxide (NaOH) solution to 7.15. The zeta potentials for 20mM, 30mM, 40mM and 60mM of HEPES solution were measured with current monitoring method to be -54.1mV, -50.3mV, -49.9mV and -48.4mV respectively. The ionic mobilities were obtained with Eq. 6.10 and are plotted in Figure 6.4 as a function of buffer concentration. The measured ionic mobilities decrease with increasing buffer solution concentration as predicted and agree very well with the experimentally obtained values by Bahga *et al* under the same buffer concentrations and pH (see Figure 6.4).

The same measurement was performed for the fluorescent solutions employed in the micromixing experiments. The buffer solution is 1mM NaHCO₃ solution (pH=7.2). The average mobility of fluorescein ion was determined to be $-2.53 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ (with standard deviation of $0.25 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$).

Figure 6.4: Comparison of measured ion mobility of disodium fluorescein in various concentration of HEPES buffer solution (pH=7.15) with experimental and theoretical values reported in literature.

6.5 Boundary Conditions

To solve the governing equation and to compare the simulated results with experiments, suitable boundary conditions have to be applied. Two out-of-phase sinusoidal AC voltages with a similar DC bias were applied at the two inlets in the experiments. The DC voltage V_{DC} applied was 270V and the AC voltages V_{AC} applied was $1/3 V_{DC}$, $2/3 V_{DC}$, V_{DC} or $4/3 V_{DC}$. Although the length of the T-junction was reduced by 5 times in the simulation, the applied voltage cannot be assumed to be $V_{DC}/5$ in the simulation. This is because the presence of constriction affects the potential distribution across the channel. The constriction length is unchanged despite the whole channel length is reduced by five times.

To establish the appropriate voltage boundary condition, a simulation for the electric potential distribution in the T-mixer with dimensions the same as the physical dimensions was performed based on the Laplace equation (Eq. 6.1). The voltages at locations A, B and C (see Figure 6.1) which corresponded to the voltages at the upper inlet, lower inlet and outlet respectively in the simulations

were determined from this simulation. The voltage at C was set to zero and the voltages at A and B were determined with reference to C. The DC voltage and AC amplitude at the inlets obtained with this simulation for various experimental conditions are listed in Table 6.1. The channel walls were assumed to be electrically insulated. Other boundary conditions are summarized in Table 6.2 .

Table 6.1 Voltage boundary condition for numerical simulation

Experimental conditions	DC bias in simulation, ϕ_{DC} (V)	AC amplitude in simulation, ϕ_{AC} (V)
$V_{AC}=1/3V_{DC}$	65.31	17.95
$V_{AC}=2/3V_{DC}$	65.31	35.91
$V_{AC}=V_{DC}$	65.31	53.87
$V_{AC}=4/3V_{DC}$	65.31	71.82

Table 6.2 Summary of boundary conditions ($\mathbf{n}$ = unit vector normal to boundary)

Variable	Condition	Boundary
Applied potential, ϕ	$\phi = \phi_{DC} + \phi_{AC}\sin(2\pi ft + \pi)$	Upper inlet
	$\phi = \phi_{DC} + \phi_{AC}\sin(2\pi ft)$	Lower inlet
	$\phi = 0$	Outlet
	$\mathbf{n} \cdot \nabla \phi = 0$	Channel wall
Velocity vector, $\mathbf{u}$ and pressure, p	$p = 0$	Inlets, outlet
	$\mathbf{u} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} \nabla \phi$	Channel Wall
Dye concentration, c_i	$c_i = 1$	Upper inlet
	$c_i = 0$	Lower inlet, outlet
	$\mathbf{n} \cdot (-D \nabla c_i - u_{mi} c_i \nabla \psi + \mathbf{u} c_i) = 0$	Channel Wall

For flow field simulation, slip velocity boundary condition is adopted here. According to the Smoluchowski equation (Eq. 6.4), zeta potential is required to compute the slip velocity. Zeta potential for the buffer solution, 1mM sodium bicarbonate (NaHCO_3) solution, was measured with current monitoring method to be -59.8mV (with standard deviation of 2.2mV), see Section 6.3. Since no external pressure was applied, pressures at the inlets and outlet were set to zero.

The normalized dye concentrations at the upper and lower inlets were set to 1 and 0 respectively for ease of mixing efficiency evaluation; these boundary conditions corresponded respectively to the dyed streams from the upper inlet and the undyed streams at the lower inlet in the experiments. Various constants employed in the simulations are listed in Table 6.3. The estimation of the mobility of fluorescein ion is detailed in Section 6.4. Diffusion constant of fluorescein in water at 21.5°C as calculated from Wilke-Chang correlation is $4.9 \times 10^{-10} \text{m}^2 \text{s}^{-1}$ (Rani *et al.* 2005).

The simulation domain was meshed with 17394 triangular elements. The electrostatic potential, ion concentration, velocity, and applied potential were discretized with second order elements while the pressure was discretized with linear elements. Convergence test for selected mesh was performed and shown in Appendix D.1.

Table 6.3: Symbols and values of constants in numerical simulation

Constant	Symbol (unit)	Value
Diffusion coefficient of fluorescein ion	$D/\text{m}^2\text{s}^{-1}$	4.9×10^{-10}
Unit electron charge	e/C	1.602×10^{-19}
Faraday constant	F / Cmol^{-1}	96485
Boltzmann constant	$k_b/\text{m}^2\text{kgs}^{-2}\text{K}^{-1}$	1.381×10^{-23}
Mobility of fluorescein ion	$u_{mi}/\text{m}^2\text{V}^{-1}\text{s}^{-1}$	-2.53×10^{-8}
Temperature	T/K	298
Relative permittivity	ε_r	80
Permittivity of free space	$\varepsilon_0 / \text{Fm}^{-1}$	8.85×10^{-12}
Density of water	ρ / kgm^{-3}	1000
Zeta potential	ζ/V	-0.0598
Viscosity of water	$\mu/\text{kgm}^{-1}\text{s}^{-1}$	8.90×10^{-4}

6.6 Dimensionless Parameters

To obtain the dimensionless parameters governing the flow, Eq. 6.1, 6.2, 6.3 and 6.6 are non-dimensionalized by introducing the following variable:

$$\phi^* = \frac{\phi}{\phi_{DC}}, \mathbf{u}^* = \frac{\mathbf{u}}{U}, t^* = \frac{Ut}{W}, \mathbf{x}^* = \frac{\mathbf{x}}{W}, p^* = \frac{p}{\rho U^2}, c_i^* = \frac{c_i}{c_{i0}} \quad (6.11)$$

where ϕ_{DC} = DC potential applied, reference velocity $U = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} E_{DC}$, with E_{DC} = DC electric field in

the downstream channel and W =width of downstream channel. E_{DC} was constant in the series of experiments conducted since the voltages applied at the two inlets had the same AC amplitude and were out-of-phase by 180° .

The governing equations can be rewritten in dimensionless form:

$$\nabla \cdot \nabla \phi^* = 0 \quad (6.12)$$

$$\nabla \cdot \mathbf{u}^* = 0 \quad (6.13)$$

$$\frac{\partial \mathbf{u}^*}{\partial t^*} + (\mathbf{u}^* \cdot \nabla) \mathbf{u}^* = -\nabla p^* + \frac{1}{Re} \nabla^2 \mathbf{u}^* \quad (6.14)$$

$$\frac{\partial c_i^*}{\partial t^*} + \mathbf{u}^* \cdot \nabla c_i^* = \frac{1}{Pe} \nabla^2 c_i^* + \alpha \nabla \cdot (c_i^* \nabla \phi^*) \quad (6.15)$$

where Reynold number $Re = \frac{\rho UW}{\mu}$, Péclet number $Pe = \frac{UW}{D}$ and $\alpha = \frac{u_{mi} \phi_{DC}}{WU}$.

For these series of simulations and experiments, $Re = 0.276$, $Pe = 501$ and $\alpha = 6.67$. Here α is a dimensionless parameter that depicts the ratio between electromigrative transport and convective transport. If α is much smaller than 1 (migration is small compared to convection), the electromigrative term can be neglected and Eq. 6.15 reduces to the normal convection-diffusion equation. However, it is noted that electromigrative term cannot be neglected here because α is much larger than 1.

Another important dimensionless parameter can be identified by non-dimensionalizing the applied potential boundary condition. The following dimensionless variables are introduced:

$$\phi^* = \frac{\phi}{\phi_{DC}}, \quad \phi_{DC}^* = \frac{\phi_{DC}}{\phi_{DC}}, \quad \phi_{AC}^* = \frac{\phi_{AC}}{\phi_{DC}} \quad (6.16)$$

where all applied electric potentials are normalized with the applied DC potential ϕ_{DC} . The dimensionless applied voltage then becomes:

$$\phi^* = 1 + \phi_{AC}^* \sin\left(\frac{\omega W}{U} t^*\right) \quad (6.17)$$

where Strouhal number $St = \frac{\omega W}{U}$ with $\omega = 2\pi f$. Here, the Strouhal numbers for AC frequencies of 2.1Hz, 4.1Hz, 6.1Hz, 8.1Hz, 12.1Hz and 16.1Hz respectively are 2.15, 4.20, 6.25, 8.29, 12.39 and 16.49.

6.7 Amplitude of Fluid Interface Oscillation

Snapshots of numerical results for mixing conditions at various AC frequencies are shown in Figure 6.5.

At the T-junction, the fluid interface oscillated due to the applied AC voltages which were out of phase by 180 degree. The fluid interface oscillation is an important process of the mixing mechanism and closely related to the mixing efficiency of the mixer. If the amplitude of oscillation is larger than the constriction width, alternate plugs of fluid are formed in the constriction. In addition, the discrepancy induced by the exclusion of the electromigration effect of fluorescent dye can be easily observed quantitatively from the prediction of the amplitude of fluid interface oscillation.

Figure 6.5: Snapshots of numerical result for mixing condition under various AC frequencies f at constant AC amplitude $V_{AC} = 4/3V_{DC}$. Color scale on right side represents normalized concentration ranging from 0 to 1.

The amplitude of the fluid interface oscillation was measured experimentally by analyzing the fluorescent images captured. The image intensity profile along the centreline of the vertical channel was obtained and normalized with the maximum and minimum intensities (see Figure 6.6). The fluid interface location is the point at which the normalized intensity is 0.5. Three experimental runs were performed for various frequencies at a constant AC amplitude $V_{AC} = 4/3 V_{DC}$. 50 images were captured for each run. A MATLAB program was written to process these images to obtain the time-dependant interface location. Curve fitting on the data was performed with the program to obtain the amplitude of fluid interface oscillation. The amplitude of oscillation was obtained from numerical results in a similar manner.

Experimental results obtained are compared with the numerical simulation results (see Figure 6.7). The numerical simulation which included the electromigration term in the Nernst-Planck equation produced oscillation amplitudes which agree very well with the experimentally obtained results. However, if the electromigration term was neglected, the amplitudes of oscillation obtained by numerical simulation were approximately double that of the experimental amplitudes. This illustrates that electromigration effect is indeed an important consideration, and that it must be included in the simulation of dye concentration distribution for obtaining reliable predictions. Neglecting the electromigration effect will result in significant error in the simulation.

Figure 6.6: (a) Experimental images at T-junction and normalization of image intensity. (b) Determination of fluid interface location by analyzing intensity profile along centerline of vertical channel.

Figure 6.7: Comparison between fluid interface oscillation amplitude obtained from experimental results, and numerical simulations with and without electromigration term in the Nernst-Planck equation. $V_{AC} = 4/3V_{DC}$.

The amplitudes of fluid interface oscillation for various AC amplitudes and frequencies were obtained from numerical simulation and normalized with the half width of the constriction (see Figure 6.8). The normalized oscillation amplitude A^* is an important variable that governs the mixing efficiency of the micromixer. Alternate plugs can only be formed in the constriction if the oscillation amplitude is larger than the half-width of the constriction ($A^* > 1$). It is noted that A^* decreases with increasing frequency and decreasing AC amplitude.

Figure 6.8: Fluid interface oscillation amplitude obtained from numerical simulation at various frequencies and AC amplitude. Oscillation amplitude is normalized with half-width of constriction. AC voltage amplitude V_{AC} is normalized with DC voltage V_{DC} .

6.8 Mixing Efficiency

The mixing efficiencies based on numerical simulations were obtained with the similar equation as the experimental values. The concentration profile of fluorescent dye along the cross-stream direction was output from COMSOL and mixing efficiency at the downstream location was calculated based on Eq. 4.1 with a program written in MATLAB. The numerical results were compared with the experimental mixing efficiency.

From Figure 6.9, it can be observed that the mixing efficiencies predicted by simulation are in good agreement with experimental results. Some discrepancies between experimental and numerical mixing efficiencies are noted at high frequency especially for high AC amplitude. This could be due to the

variability of the constriction width due to micro-fabrication error and the uncertainty of the value of diffusion constant for the fluorescent dyes adopted from the literature, which could be slightly different from the actual value. However, this does not affect the overall trend of the results and analysis on mixing mechanism. Detailed error sensitivity analyses are contained in Section 7.10 to address these discrepancies.

Mixing efficiency with just DC voltage applied was only 38%. Mixing efficiency increased as two out-of-phase AC voltages were applied in addition to the DC voltage. Maximum mixing efficiencies as high as 86% were achieved in the frequency range of 4Hz to 6Hz. Increasing the frequency beyond 8Hz gradually reduced the mixing efficiency. Applying higher AC amplitude yielded higher mixing efficiencies for all frequencies.

Figure 6.9: Mixing efficiencies obtained at 1.2mm downstream from T-junction with numerical simulation (lines) and experiments (markers) at various AC frequencies and amplitude. AC amplitude V_{AC} is normalized with DC voltage V_{DC} .

6.9 Contact Area for Diffusive Mixing

The improvement of mixing efficiency is attributed to the increase of contact area between the two fluids due to the formation of crescent pattern after the constriction due to the sudden expansion of the channel. The increase of contact area was quantified based on numerical results. Since the numerical simulation was performed in a two dimensional space, the contact area could be represented by contact length instead. Numerical simulation is advantageous in this analysis because the results can be output at any particular time of interest to show the images at different phases of the cycle. Analysis of

experimental results on this aspect was restricted by the limited capturing speed (frame rate) of the CCD camera.

The contact lengths within the confined area shown in Figure 6.10: were obtained at different phases of the cycle. To obtain a clear outline of the contact length, post-processing of the numerical results was performed. A threshold of normalized concentration $c_f=0.5$ was set to reveal the contact line of the two fluids. The contact lines were measured with image processing software, ImageJ and normalized with the width of the channel. Figure 6.10: shows the contact length obtained at various AC frequencies and amplitudes.

Figure 6.10: Simulation results showing flow pattern. Threshold of $c_f=0.5$ was set to show clearly contact line between the two fluids after expansion process at exist of constriction. Arrows show velocity vector of flow. Total contact length within 200μm x 200μm area was measured.

If only a DC voltage is applied, mixing only occurs through diffusion at the straight interface at the middle of the two streams. Applying two out-of-phase AC voltages generates contact interfaces

which span across the entire width of the channel due to the formation of crescent-shaped pattern after the constriction. The contact length is increased by 3.5 to 4.5 fold through this simple mechanism at the frequency range of 4Hz to 8Hz. Higher contact length allows more mixing through diffusion across the interface. If the frequency is below 2Hz or beyond 16Hz, the normalized contact lengths decrease to 2 only (see Figure 6.11a). From Figure 6.11b, the contact length is increased with higher AC amplitude. This is because higher AC amplitude results in larger crescent or wavy patterns that span across the whole width of the channel, thus creating larger contact area.

Figure 6.11: Two fluid stream contact length in an area of $200\mu\text{m} \times 200\mu\text{m}$ after constriction, obtained from numerical simulation for (a) various AC frequencies at $V_{AC} = 4/3 V_{DC}$ (b) various AC amplitudes V_{AC} (normalized with DC voltage V_{DC}) at AC frequency $f = 6.1\text{Hz}$ for different phases in one cycle. Contact length is normalized with width of the channel.

6.10 Effect of Phase Difference

In the experimental investigation, the phase difference between the two applied sinusoidal voltages is fixed at 180° . Numerical simulations for the phase difference of 90° and 45° phase difference were performed at various frequencies with constant AC and DC electric fields ($E_{AC} = E_{DC} = 258\text{Vcm}^{-1}$). The mixing efficiency with 180° phase difference is significantly higher as compared to the case of 90° and 45° phase difference, at all frequencies (see Figure 6.12). The high mixing efficiency is attributed to the higher fluid fluctuation amplitude with high phase difference between the input voltages. For example, at $f=4\text{Hz}$, the fluid interface oscillation amplitude for 180° , 90° and 45° phase difference are $83.6\mu\text{m}$, $57.4\mu\text{m}$ and $29.6\mu\text{m}$ respectively. Higher oscillation amplitude leads to the formation of symmetrical crescent after the constriction and yields homogenous mixture of fluids (see Figure 6.13).

Figure 6.12: Mixing efficiency obtained from numerical simulation at various phase difference of sinusoidal applied voltages. $V_{AC}=V_{DC}$.

Figure 6.13: Snapshots of numerical result for mixing condition under applied sinusoidal voltages with (a) 45° (b) 90° and (c) 180° phase difference. $f=4\text{Hz}$, $V_{AC}=V_{DC}$. Color scale on right side represents normalized concentration ranging from 0 to 1.

Concurrently, under sinusoidal driving pressures, Sun and Sie (2010) investigated the mixing efficiency in a micromixer which is geometrically similar to the micromixer proposed in this thesis under sinusoidal driving pressures¹ (see Section 2.8 of Chapter 2). The experimentally obtained mixing efficiency for the case of 90° phase difference was reported to be higher than the case of 180° phase difference. This seemingly contradicting result can be explained by the significant difference in Reynolds numbers in their investigations. The Reynolds number in the experimental investigation by Sun and Sie

¹ The results from Sun and Sie [Sun, C.L. and Sie, J.Y. (2010). *Microfluidics and Nanofluidics* 8(4): 485-495] and the current investigation [Lim, C.Y., Lam, Y.C. and Yang, C. (2010). *Biomicrofluidics* 4(1):014101] were published in the same year on different journals.

ranged from 15 to 40 while the Reynolds number in this simulation was much less than 1 ($Re = 0.276$). Sun and Sie found that the mixing efficiency enhancement at 90° phase difference was attributed to the instability induced by the varying throat pressure (the pressure at the entrance of the constriction). This variability of throat pressure was absent in the case of 180° phase difference because the sinusoidal component of the driving pressure cancelled out each other.

It has to be highlighted here that in the numerical and experimental investigations conducted in this study, the flow is stable due to the low Reynolds number (Figure 6.14). Inertia-induced flow instability was absent in the micromixer, even with oscillating flow. Normally under these conditions, good mixing will be difficult to be achieved. The achieved mixing enhancement was attributed to the significant increase of contact surface downstream of the constriction which facilitated the diffusion process. As mixing efficiency was determined by the homogeneity of the solute across the channel, input signals with 180° phase difference which yielded more symmetrical crescent shaped patterns would lead to a higher mixing efficiency, as compared to 45° and 90° phase differences.

Figure 6.14: Critical Reynolds number of flow instability versus half-angle for Jeffery-Hamel flow. Dot symbols represent the experimental results conducted by Sun and Sie (Sun and Sie 2010).

6.11 Error and Sensitivity Analyses

Detailed error analyses were performed to investigate the discrepancy between numerical and experimental results, especially at high AC amplitudes and frequencies. In particular, the sensitivities of the simulation results to the uncertainties in the width of constriction, diffusion coefficient and fluorescein mobility were examined. The possible error induced by neglecting electromigration due to the wall electric potential will also be discussed.

The average width of the constriction was measured to be $40\mu\text{m}$, with a standard deviation of $3\mu\text{m}$ based on 14 microchannels. However, the actual width of the constriction varies from $31\mu\text{m}$ to $45\mu\text{m}$ due to variations induced during micro-fabrication. To investigate the uncertainty induced by assuming an average constriction width of $40\mu\text{m}$ in the simulations, the simulation for the case of $V_{AC}=4/3V_{DC}$ was repeated with minimum and maximum constriction widths of $31\mu\text{m}$ and $45\mu\text{m}$. It can be observed that the mixing efficiencies at high frequency are more sensitive to the variability of constriction width as compared to low frequency (see Figure 6.15a). If the constriction width was $31\mu\text{m}$ instead of the average $40\mu\text{m}$, the mixing efficiency at $f=16\text{Hz}$ increases by 9.1% whereas the mixing efficiency at $f=4\text{Hz}$ only increases by 4.7%.

The mixing efficiencies results are sensitive to the variation of constriction width, especially at $f=12\text{Hz}$ and $f=16\text{Hz}$ because the fluid interface oscillation amplitudes are very small. The ratio between the oscillation amplitude and the constriction width is an important parameter that governs the mixing efficiency. Small deviations of constriction width from the average value ($+5\mu\text{m}$ to $-9\mu\text{m}$) induce relatively large error in the simulated mixing efficiency because the oscillation amplitude at 12Hz and 16Hz are only $27\mu\text{m}$ and $17\mu\text{m}$ respectively.

Diffusion coefficient of fluorescein ion has been measured by a few researches (Zero *et al.* 1983; Culbertson *et al.* 2002; Du *et al.* 2006; Petrásek and Schuille 2008). The reported value of diffusion

coefficient varies from $4.25 \times 10^{-9} \text{m}^2 \text{s}^{-1}$ to $6.3 \times 10^{-9} \text{m}^2 \text{s}^{-1}$. The value of diffusion coefficient as calculated with Wilke-Chang correlation is $4.9 \times 10^{-9} \text{m}^2 \text{s}^{-1}$ (Rani *et al.* 2005) which is the value adopted in the simulation. The simulations were repeated with the two extreme values of diffusion coefficients reported in the literature (see Figure 6.15b). If the higher value of diffusion coefficient ($6.3 \times 10^{-9} \text{m}^2 \text{s}^{-1}$) was adopted in the simulation, the mixing efficiencies for $f=0\text{Hz}$ and $f=16\text{Hz}$ increase by 6.6% and 5.2% respectively while the mixing efficiency for $f=6\text{Hz}$ only increases by 1.7%.

The mixing efficiency is sensitive to diffusion coefficient at low (0Hz to 2Hz) and high frequencies (12Hz to 16Hz) while the mixing efficiency is not affected significantly at the intermediate frequency range (4Hz to 8Hz). This is due to the fact that for intermediate frequency range, the solutions are almost completely mixed at the downstream cross section where the mixing evaluation is performed (as indicated by the high mixing efficiency for $f=4\text{Hz}$ to $f=8\text{Hz}$). As such, the concentration gradient has been reduced significantly and the diffusion process becomes less dominant. This translates to the mixing efficiency being less sensitive to the uncertainty in diffusion coefficient. In contrast, for the low and high frequencies, the mixing efficiencies are low and significant concentration gradient still persists across the channel. Therefore, the diffusion process remains significant in contributing to mixing efficiencies. Thus, mixing efficiencies are sensitive to the uncertainty in diffusion coefficient at low and high frequencies.

The measured values of fluorescein mobility in the literature can vary significantly from $-0.3 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ to $-3.7 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ (Cheng *et al.* 1990; Whang and Yeung 1992; Kim *et al.* 2006; Whiting *et al.* 2008) at room temperature depending on pH, concentration and type of buffer solution. However, since the experimental conditions of the measured mobility value in the literature are different from our experiments, these values were not adopted in the simulation. Instead, simulations were performed with the theoretically predicted mobility of $-3 \times 10^{-8} \text{m}^2 \text{V}^{-1} \text{s}^{-1}$ (Bahga *et al.* 2010) for comparison with the

simulation results with the measured mobility ($-2.53 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ in 1mM NaHCO_3 buffer solution at pH = 7.2) in Section 6.4. The mixing efficiencies at low frequencies (0Hz and 2Hz) are sensitive to the variability of the mobility value. However, the mixing efficiencies at higher frequencies (4Hz and above) do not vary significantly with the variability of mobility value (see Figure 6.15c). Therefore, the discrepancies observed at high frequencies simulation are not likely to be caused by the uncertainty in mobility measurement.

The effect of wall potential on the fluorescent dye has been neglected in the simulation. The possible error induced is examined here. To model the electromigration of fluorescein ion due to wall potential, Poisson-Boltzmann equation has been introduced to describe the electric potential, ψ of the charge wall.

$$\psi = \frac{2c_0 F}{\varepsilon_r \varepsilon_0} \sinh\left(\frac{e\psi}{k_b T}\right) \quad (6.18)$$

where c_0 = bulk concentration of ion, F =Faraday constant, e =unit electron charge, k_b = Boltzmann constant, T = absolute temperature. The wall potential is added to the electromigration term in the Nernst-Planck equation through the principle of superposition of electric potential.

$$\frac{\partial c_i}{\partial t} + \nabla \cdot [-D \nabla c_i - u_{mi} c_i \nabla(\phi + \psi)] = -\mathbf{u} \cdot \nabla c_i \quad (6.19)$$

Figure 6.15: Simulation results showing effect of uncertainties in determination of (a) constriction width (b) diffusion coefficient (c) fluorescein ion mobility on mixing efficiency at various AC frequencies for $V_{AC} = 4/3 V_{DC}$. Simulation results are shown with lines while experimental results are shown with triangular symbols.

The simulation domain was meshed with 29,933 triangular elements. Additional 12 layers of rectangular elements (12,348 elements in total) were added at the wall boundaries to capture the steep changes of electric potential and ion concentration in the EDL. The rectangular elements were 50nm thick at the wall boundaries and the thickness of the elements in the next layer increased by 1.2 times (in the direction away from the wall). The simulations of $f=4\text{Hz}$ and $f=16\text{Hz}$ with $V_{DC}=4/3V_{AC}$ have been repeated with consideration of the electromigration due to wall potential. The ion concentrations at 1.2mm downstream obtained from simulations with and without wall potential are compared in Figure 6.16. The curves for both cases overlapped each other across the width of the channel in the bulk region. The only difference is that the simulations with wall potential show that the ion concentration increased sharply near the edges of the channel. However, since the EDL region is in the order of nanometers, the effect of wall potential on the overall mixing efficiency which is evaluated across the entire width of the channel (200 μm) is negligible. Therefore, the omission of electromigration due to wall potential in the simulations performed in this study has resulted in computation efficiency without sacrificing accuracy.

Figure 6.16: Simulation results showing ion concentration at $f=4\text{Hz}$ and $f=16\text{Hz}$ for $V_{AC} = 4/3 V_{DC}$. Dash curves show simulation results performed with ion electromigration due to channel wall potential while solid curves show simulation results neglecting ion eletromigration due to channel wall potential. Cross-stream position is normalized with channel width.

6.12 Summary

Numerical investigations have been performed on the mixing mechanism of a micromixer based on periodic electroosmotic flow through a constriction. Dimensionless analysis and numerical simulation reveal that electromigration of charged fluorescent dye species is an important phenomenon which cannot be neglected as it can affect the flow behavior significantly. Failure to consider electromigration in the simulation could result in significant discrepancies (as large as 50%) between experimentally observed and numerically predicted fluid interface oscillation amplitudes.

Important factors that govern the mixing efficiency of the micromixer such as fluid interface oscillation amplitude and contact length between the two fluid streams were quantified based on experimental and numerical results. The proposed micromixer can effectively increase the contact area between the two fluids through the expansion of alternate fluid plugs from a constriction. Optimum Strouhal number which maximizes the contact area ranges from approximately 4.2 to 8.3. Larger AC amplitude as compared to the DC driving voltage was also found to produce higher contact area. The increase of contact area between the two fluids facilitates mixing through diffusion as they are convected downstream. Based on the numerical model, applied AC amplitude and frequency can be optimized to obtain the desired mixing efficiency.

Error and sensitivity analyses have been performed to investigate the sensitivity of the mixing efficiency to the uncertainties in constriction width, diffusion coefficient and fluorescein ion mobility. Mixing efficiency is found to be more sensitive to the uncertainty of constriction width at high frequency. Uncertainty of diffusion coefficient affects the mixing efficiency more significantly at low and high frequencies, as compared to the intermediate frequencies (4Hz to 8Hz). The uncertainty of fluorescein ion mobility affects the mixing efficiency significantly at low frequency.

Zeta potential is an important parameter which is required for the modeling of the flow behavior in an electrokinetically driven micromixer. The zeta potential of the buffer solution was measured with the conventional current monitoring method. The addition of a small amount of fluorescent dye such as disodium fluorescein and Rhodamine B was verified to have a negligible effect on the zeta potential.

A novel method was proposed and demonstrated for the measurement of the mobility of the fluorescent dye. In a T-channel, oscillation of fluid interface induced the formation of crescent-shaped patterns through a constriction. The movement of the crescent downstream was captured with CCD

camera. Consecutive images of the moving crescent were then analyzed and the mobility of the fluorescent dye was calculated by knowing the electroosmotic flow velocity of the bulk solution. The measured mobilities of fluorescein ion in the buffer solutions of various concentrations agree well with the theoretical and experimental values reported in the literature.

Chapter 7 Direction Dependence of Displacement Time for Two-fluid Electroosmotic Flow

7.1 Introduction

In flow simulation of an electrikinetically driven micromixer, zeta potential is often required to be specified as the boundary condition. One of the popular methods to measure zeta potential is current monitoring technique which involves the displacement flow of two fluids with small concentration difference. Displacement time which is the time required for a fluid to fully displace another fluid that resides in the microchannel initially can be related to the zeta potential. There is experimentally observed anomaly in such flow, namely the displacement time is flow direction dependent. In other words, the time required for a low concentration solution to displace a high concentration is not the same as the time required for a high concentration solution to displace a low concentration solution. Hitherto, no theoretical or numerical studies have been conducted to explain this hysteretic phenomenon.

This chapter presents an in-depth investigation on the displacement time difference in two fluids electroosmotic displacement flow in glass and PDMS microchannels. A numerical model for two fluids displacement electroosmotic flow is introduced to describe and explain the direction dependence of the displacement time.

7.2 Review of Two-fluid Displacement Flow and Frustrated Flow

Two-fluid displacement flow is commonly encountered in various microfluidic systems and experiments. Current monitoring technique is a popular method to measure zeta potential in a displacement flow involving two solutions with a small difference in concentration, typically 5%(Huang *et al.* 1988). When a solution is displacing another solution with a lower or higher concentration, the electrical resistance in the microchannel changes. This change induces an observable electrical current change. When the solution has fully displaced another solution, the current reaches a steady value because the resistance in the microchannel is now constant (see Section 2.10). By monitoring the current changes in real time, the time for the current to reach a steady value (i.e. the displacement time) can be determined. The zeta potential can then be calculated through the displacement time with the Smoluchowski slip velocity equation. The idea of utilizing displacement flow of solutions with small (5 to 7.5%) concentration difference has been adapted and improved by other researchers (Sze *et al.* 2003; Almutairi *et al.* 2009; Vishal *et al.* 2009) to measure the zeta potential of a channel wall.

Displacement flow with a larger concentration difference has been reported. Mampallil *et al.*(Mampallil *et al.* 2010) demonstrated that the surface charge of glass/PDMS channel wall can also be obtained with two-fluid displacement flow, with the ratio of concentration between the two solutions ranged from 2:1 to 10:1. The surface charge was assumed to be constant regardless of the concentration of the solutions. All displacement flows measured were uni-directional with high concentration solution displacing low concentration solution. Recently, Tang *et al.*(Tang *et al.* 2010) performed a theoretical investigation on electroosmotic displacement flow involving two or more fluids. Asymptotic cases where a fluid is displaced by a very high or a very low conductivity solution have been considered. Hitherto, for the displacement flow involving solutions with a large concentration difference, there are no systematic investigations on the difference in displacement

time between the two different displacement directions.

Gan *et al* (2006) derived the expression for the displacement time for two fluids electroosmotic flow and plotted the displacement curve for flows in both directions. The displacement time was obtained by solving Navier-Stokes equation for fluid flow field and Poisson equation for electric field. According to the analysis, *the displacement times for both directions are exactly the same*. Hence, EOF hysteresis effect is not expected according to the conventional formulation for electroosmotic flow because the ion concentration in the EDL layer is assumed to be unaffected by the flow field. Therefore flow velocity and displacement time are only affected by the composition of bulk concentration and the corresponding zeta potential.

To illustrate the expected flow behaviors based on conventional theory or intuitive reasoning, comparison between snapshots of the electroosmotic displacement flow of two fluids (fluid A and B) from two opposite directions was illustrated in Figure 7.1. It should be noted that, each snapshot for the flow in one direction has an exact match with another snapshot for the flow in the opposite direction. In other words, if there are no arrows showing the flow directions, one will not be able to differentiate the two series of flow snapshots. Conventional theory thus predicts that the bulk concentration composition at each snapshot and the development of zeta potential distribution for flows in the opposite directions are *identical* but their orders of occurrence are simply *reversed* (see Figure 7.1). Therefore, the displacement time should be exactly identical and should *not* be dependent on the flow direction. However, this intuitive conclusion is shown to be incorrect through experiments in Section 7.6.

Figure 7.1: Snapshots for fluid composition (A and B) in two-fluid electroosmotic displacement flow in opposite directions. (a) Fluid B displaces Fluid A. (b) Fluid A displaces Fluid B. Horizontal arrows shows the flow directions and the vertical arrows shows the time order for these snapshots.

The concentration mismatch between two fluids causes internal pressure gradient which generates a frustrated flow. The frustrated flow profile is a superposition of a plug-like profile of EOF and parabolic profile of pressure driven flow (Chang and Yeo 2010). Frustrated flow has also been reported as a result of non-uniform zeta potential on channel wall (Herr *et al.* 2000) or pH gradient (Minerick *et al.* 2002). However, hitherto there is no study on the *effect of the flow profile distortion, the induced convection and diffusion effects on the ion distribution in EDL layer*. This effect which is hypothesized to be the potential cause of EOF hysteresis effect will be investigated through numerical simulations in this chapter.

7.3 Review of Electroosmotic Flow Model

Conventional electroosmotic flow model can be categorized into three major groups, namely slip-velocity model, Poisson-Boltzmann (PB) model and Poisson-Nernst-Planck (PNP) model. Slip velocity model is the simplest model whereby the velocity variation in the thin EDL is neglected. The velocity at the wall of the microchannel is approximated by the Smoluchowski slip velocity, v_{eo} :

$$v_{eo} = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\mu} E \quad (7.1)$$

This model has been adopted by various researchers (Hu *et al.* 2003) and the micromixing simulation in Chapter 6 due to its simplicity in computation.

More sophisticated models that take into account the change of variables in the EDL are the Poisson-Boltzmann (PB) model and the Nernst-Planck (NP) model. PB model assumes that the positive and negative ion distributions at the vicinity of the wall are given by Boltzmann distribution:

$$c_{\pm} = c_o \exp\left(\mp \frac{e\psi}{k_b T}\right) \quad (7.2)$$

where $c_{\pm}$ =concentration of positive or negative ion, c_o =bulk concentration of solution, e =elementary charge, ψ =static electric potential, k_b =Boltzmann constant and T =temperature. After obtaining the ion distribution, Navier-Stokes (NS) equation can then be solved separately to obtain the velocity field (Arulanandam and Li 2000; Tang *et al.* 2004; Yang and Liu 2008).

In contrast, PNP model describes the ion distribution in the liquid according to the Nernst-Planck equations for positive and negative ion species:

$$\frac{\partial c_{\pm}}{\partial t} + \nabla \cdot [-D_{\pm} \nabla c_{\pm} - u_{m\pm} c_{\pm} \nabla(\phi + \psi)] = -\mathbf{u} \cdot \nabla c_{\pm} \quad (7.3)$$

where $D_{\pm}$ =diffusion coefficient of positive or negative ion, $u_{m\pm}$ =mobility of positive or negative ion, F =Faraday constant, ϕ =applied electric potential and $\mathbf{u}$ =fluid velocity. The Nernst-Planck equation

has to be solved simultaneously with the NS and Poisson equations to obtain the flow field because the electric potential, positive/negative ion concentration and flow velocity are strongly coupled together.

For the slip velocity model, the boundary condition at the wall is set to a slip velocity, which is proportional to a prescribed zeta potential value. It does not rely upon nor provides any information on ions distributions, including those next to the wall. Thus, for a situation where the ions (and charges) distributions next to the wall are varying, it could not be expected to provide accurate predictions. Flows with zeta potential which varies along the channel can be modelled with a modified slip velocity model (Chang and Yeo 2010). However, the derivation of the slip velocity expression requires the Debye-Hückel approximation which is only valid for small surface potential and external perturbations such as diffusion or convection, and their effects on the ion distribution in the EDL are not taken into account. It is also not applicable to nanochannel where overlapping of EDL is expected.

PB and PNP models define the boundary of the model at the shear plane of the EDL, which is the plane at which the fluid particles start moving. Therefore, a non-slip condition for velocity is set at the wall boundary. The boundary conditions at the channel wall for potential distribution and ion concentration are normally specified in terms of the zeta potential of the solution (Fu *et al.* 2003; Bhattacharyya and Nayak 2009; Park and Choi 2009). During the displacement flow of two solutions, diffusion occurs at the interface of these two solutions and therefore the zeta potential (which is a function of the concentration of the solution) in this interface region cannot be specified explicitly. Internal pressure gradient generated (Herr *et al.* 2000) at the interface might also affect the flow field and the ion distribution near the wall, thus further complicating the specification of boundary conditions as required by PB and PNP models.

Alternatively, instead of specifying the boundary condition at the wall, the boundary

condition along the line of symmetry (at the bulk of the fluid) can be defined in terms of the bulk concentration of the solution which reflects the characteristics at the channel wall (Kwak and Hasselbrink Jr 2005; Craven *et al.* 2008). However, the boundary condition at the bulk cannot be clearly specified to characterize the conditions at the wall around the vicinity of the interface for the two flowing solutions: there are two characteristic concentrations to start with and the conditions are not at steady state due to diffusion and convection induced by internal pressure gradient at the interface.

Therefore, modelling any flow phenomena which involve perturbation of the ion distribution in EDL that would lead to changes in zeta potential is a challenging task. This perturbation can be expected for flows involving dissimilar fluids and flows in nanochannels.

7.4 Materials and Equipments

In this study, 1mM KCl solutions were prepared by dissolving KCl salt (Merck) in deionized water. KCl solution of 0.2mM, 0.5mM, 0.7mM and 0.95mM were prepared by diluting the 1mM KCl solutions accordingly. The properties of all solutions were measured with a conductivity meter (IONCheck 65, Radiometer Analytical) and pH meter (AccumetAR20, Fisher Scientific). The measured conductivities for 0.2mM, 0.5mM, 0.7mM, 0.95mM and 1mM KCl solutions were 31.8 μ S/cm, 74.3 μ S/cm, 104.3 μ S/cm, 137.6 μ S/cm and 147.0 μ S/cm respectively. Measured average pH for all solutions was 5.5.

The microchannel employed was polyimide coated fused silica micro-capillary (Polymicro Technologies) with a nominal diameter of either 20 μ m, 75 μ m, 100 μ m or 150 μ m. Micro-capillaries with length of 6cm, 8cm and 10cm were measured and cut with Shortix Column Cutter (SGT Ltd). Two Teflon reservoirs were fabricated with diameter and depth of 2cm. A microcapillary was connected between these two reservoirs. The diameters of the reservoirs were sufficiently large to

prevent noticeable liquid level changes during the course of the experiment. This will minimize the back pressure arising from the difference of liquid level in the reservoirs (Yan *et al.* 2007).

Besides the glass micro-capillaries, rectangular polydimethylsiloxane (PDMS) microchannels were also employed as a comparison with the glass micro-capillaries. Each of the PDMS microchannels is 5.5cm long, and the width and depth of the microchannel are 100 μm and 45 μm respectively. The PDMS microchannels were fabricated through soft lithography technique with a negative photoresist SU-8 master which has the protruding microchannel pattern with the required dimensions on a silicon wafer. The PDMS was first prepared by mixing the base and the curing agent at 10:1 weight ratio. Half of the mixture was then poured onto the master while the remaining was poured into a petry dish. The master formed the three walls of the rectangular microchannel in PDMS while the PDMS slab from the petry dish provided the fourth surface required to form a closed microchannel. They were cured at 80°C for 1 hour. The cured PDMS slab from the petry dish was bonded with oxygen plasma treatment to a piece of glass slide to increase the overall rigidity of the PDMS microchannel. The cured PDMS channel on the master was peeled and holes were punched to form the inlet and outlet of the channel. The PDMS channel was then bonded to the PDMS slab with oxygen plasma treatment to form a closed four-walled PDMS microchannel. The reservoirs (diameter of 2cm and height of 0.5cm) were fabricated from PDMS and attached to the inlet and outlet of the microchannel.

A high voltage power supply (CZE1000R, Spellman) was employed to provide the electric field for inducing electroosmotic flow. The current was monitored by a picoammeter (Keithley 6485) which was connected in series to the microcapillary (see Figure 7.2). A Labview program was written to control the two devices and to record the voltage and current readings through a data acquisition card (PCI-6052E, National Instrument).

Figure 7.2: Schematic diagram of experimental setup for current monitoring method

7.5 Experimental Methods

All glass micro-capillaries were flushed with acetone followed by deionized water and KCl solution before use. Flushing was performed through a syringe filled with the required fluid that was connected to the micro-capillary through a silicone tubing. PDMS microchannels were flushed with KCl solution only. For a low concentration fluid displacing a high concentration fluid, after flushing, the microchannel was filled with 1mM KCl solutions. Reservoir 2 was also filled with 1 ml of 1mM KCl solution (see Figure 7.2). Reservoir 1 was then filled with KCl solution with a lower concentration (0.2mM, 0.5mM, 0.7mM or 0.95mM). Subsequently, voltages of 500V, 1000V or 1500V were applied across the two reservoirs to generate electroosmotic flow across the glass microcapillary. For the PDMS microchannel, a voltage of 330V was applied. Similarly, the displacement flow of a high concentration fluid displacing a low concentration fluid was conducted in a similar fashion. In this case, the lower concentration KCl solutions were filled in the microchannel and reservoir 2 while 1mM KCl solution was filled in reservoir 1 instead.

Experiments were conducted with various concentration ratios between the two solutions, lengths and diameters of microchannel. Each set of parameters was conducted at least five times to

obtain consistent and reliable results.

The effect of Joule heating in our investigation can be neglected. It is caused by volumetric heating when an electric field is applied across a conductive media such as electrolyte. However, if the concentration and conductivity of the electrolyte are low, the temperature rise due to Joule heating is negligible (Tang *et al.* 2006). A conservative estimate of temperature rise from Joule heating can be derived from the energy balance between the energy generation, E_g , and the energy storage, E_{st} , in the liquid as

$$E_g = E_{st}$$

$$\frac{V^2}{R_L} \Delta t = \rho V_c C_p \Delta T \quad (7.4)$$

where ΔV is the applied voltage difference, Δt is the total time the voltage is applied, R_L is the electrical resistance per unit volume of the liquid, ρ is the liquid density, V_c is the total liquid volume, C_p is the specific heat of the liquid, and ΔT is the average temperature change of the liquid (Arulanandam and Li 2000). The worst case scenario given by the parameters investigated (applied voltage = 1500V, solution concentration = 1mM, diameter of capillary = 20 μ m, length of capillary=8cm) is an estimated temperature rise of 0.27°C only, which is negligible.

Another experimental issue worth mentioning is the electrokinetic instability. Lin *et al* (2004) observed electrokinetic instability when the applied voltage is 500V/cm and the conductivity ratio between the two solutions is 10:1. This instability effect is not applicable in the present investigation because the electric fields applied in the experiment, which ranges from 125V/cm to 375V/cm are much lower than the threshold voltage to cause instability. Furthermore, the maximum conductivity ratio between the two solutions employed in the experiment does not exceed 5:1.

7.6 Experimental Results

Figure 7.3 shows an example of the current-time curve for the displacement flow of 1mM and 0.2mM KCl solutions in the glass micro-capillary. The case of 1mM KCl displacing 0.2mM KCl is depicted by the ascending curve while the case of 0.2mM KCl displacing 1mM KCl is represented by the descending curve. The time for the current to reach a steady value is the time required for the fluid from reservoir 1 to fully displace the fluid in the microcapillary. It can be observed clearly that the displacement times for the two cases were indeed different.

Figure 7.3: Current-time curve for displacement flow of 0.2mM and 1mM KCl solutions in glass microcapillary.

Fig. 3 shows the displacement time for different pairs of solutions under various voltages for glass micro-capillary of 100 μm in diameter and 8cm in length. Student's t-test was performed on the data to examine if the mean displacement times of these two different directions are significantly different. Let T_{HL} = displacement time for high concentration solution displacing low concentration solution and T_{LH} = displacement time for low concentration solution displacing high concentration solution. The null hypothesis H_0 states that T_{LH} is not larger than T_{HL} while the alternative hypothesis H_1 states that T_{LH} is larger than T_{HL} . The t-score can be calculated as

$$t_s = \frac{\bar{T}_{LH} - \bar{T}_{HL}}{\sqrt{\frac{s_{LH}^2}{n} + \frac{s_{HL}^2}{n}}} \quad (7.5)$$

where $\bar{T}_{LH}$ = sample mean of T_{LH} , $\bar{T}_{HL}$ = sample mean of T_{HL} , s_{LH} = sample standard deviation of T_{LH} , s_{HL} = sample standard deviation of T_{HL} and n = number of sample.

Figure 7.4: Displacement time for 1mM KCl with (a) 0.2mM, (b) 0.5mM, (c) 0.7mM and (d) 0.95mM KCl for flows in both directions in glass micro-capillary with diameter of 100 μ m and length of 8cm under applied voltage of 1000V. Error bars indicate the standard deviations.

Table 7.1 shows the t -score calculated from the experimental data. With 5 samples from each group of data, the degree of freedom is 8. The critical t -score for a significance level of 0.005 in a one-tailed test is 3.36. With the exception of 0.95mM and 1mM solution pair, all solutions pairs show t -scores higher than the critical t -score. This implies that the probability for the observed time difference to be caused by sampling or random error is very small (less than 0.5%) for the displacement flow of 0.2mM, 0.5mM and 0.7mM with 1mM. Therefore, the null hypothesis H_0 is rejected in favor of the alternative hypothesis H_1 for these three pairs of solutions.

Table 7.1: t -score for displacement time of various solutions pairs at 3 voltages applied in glass micro-capillary.

Solution pairs	Voltage applied		
	500V	1000V	1500V
0.2mM & 1mM	18.87	25.35	13.08
0.5mM & 1mM	6.11	11.09	13.07
0.7mM & 1mM	4.46	5.85	8.95
0.95mM & 1mM	1.30	3.20	1.06

For a better comparison of time differences between different pairs of solutions, percentage time difference is calculated as $(T_{LH} - T_{HL})/T_{HL} \times 100\%$ and plotted with respect to percentage concentration difference, see Figure 7.5a. It is observed that the percentage displacement time difference increases with higher concentration difference between the two solutions. Each pair of solutions show approximately similar percentage time difference at the three voltages applied. Therefore, the percentage time difference is not dependant on the applied voltages. The average percentage time differences for 0.95mM, 0.7mM, 0.5mM and 0.2mM KCl with 1mM KCl are 4.3%, 13.4%, 19.4% and 28.4% respectively.

Figure 7.5: Percentage time difference between displacement time in both directions for KCl solution with various percentage of concentration differences at various (a) voltages (with micro-capillary length of 8cm and diameter of 100μm), (b) micro-capillary diameters (length was fixed at 8cm), and (c) micro-capillary lengths (diameter was fixed at 100μm).

These results also show that in a conventional current monitoring method which involves displacement flow of solution with 5% concentration difference, the difference in displacement time between the two different flow directions is not significant (less than 5%). Therefore, zeta potential which is calculated based on displacement time should be almost similar regardless of flow directions in this case.

Displacement flows with three pairs of solutions were also performed with glass

microcapillaries of different lengths and diameters with applied voltage fixed at 1000V. Similar trend which shows displacement time difference increases with increasing concentration difference was obtained, see Figure 7.5b and c. Despite changing the diameter and length of the micro-capillary, the percentage displacement time difference is approximately the same for a particular concentration difference. Therefore, diameter and length of micro-capillary do not seem to affect the time difference significantly.

The experimental results based on displacement flow with similar solution pairs in PDMS microchannels is shown in Figure 7.6a. 6.5% and 13% displacement time differences were observed for 50% and 80% concentration differences respectively. However, the time difference was significantly lower than glass microcapillary at similar concentration difference (see Figure 7.6b). Contrary to glass micro-capillary, the displacement time in PDMS channel from opposite directions does not differ significantly at 30% concentration difference. This shows that the observed time difference is dependant on the material of the channel wall.

Figure 7.6: (a) Displacement time in PDMS microchannel with KCl solutions of various percentage concentration differences under applied voltage of 330V over channel length of 5.5cm (b) Comparison between percentage time difference for PDMS channel and glass micro-capillary (6cm) at various concentration difference.

7.7 Numerical Model

Hitherto, no numerical and theoretical studies have been performed to study the displacement time difference reported in Section 7.6. Conventional models which prescribe a constant zeta potential or bulk concentration cannot describe adequately the two fluid displacement flow because they do not consider the changes of ion concentration near the EDL due to diffusion and convection. When one fluid is displacing another fluid under electromostic flow, the zeta potential along the microchannel and ion distribution are constantly changing due to the dissimilar concentration between the two fluids with constant perturbation from diffusive and convective effects. Analytical solution based on conventional Navier-Stokes equation and Poisson equation cannot be expected to show this hysteresis effect (Ren *et al.* 2001; Ren *et al.* 2003; Gan *et al.* 2006). Numerical models (Schonfeld *et al.* 2009) based on similar assumptions also fails to predict the EOF hysteresis effect. Therefore, a new numerical scheme with boundary conditions which does not specify *a priori* zeta potential and ion distribution in EDL were formulated in this investigation.

A numerical simulation with Finite Element Method (FEM) was conducted based on the PNP model with modified boundary conditions to explain the displacement time difference phenomenon in two fluid displacement flow. The model is implemented on COMSOL Multiphysics software. The simulation is conducted based on a straight cylindrical microchannel with diameter of 20 μm and length of 160 μm . The fluid flow is assumed to be axisymmetrical about the centre axis of the cylindrical channel and therefore, an axisymmetric analysis is conducted, see Figure 7.7.

Figure 7.7: Simulation domain and coordinate system for axisymmetric analysis.

To capture the fine details necessary to describe the two fluid displacement flow, a set of strongly coupled governing equations for fluid flow, ion distributions, wall potential and applied electric potential have to be solved simulatenously. Therefore, a large amount of computer memory is required. Thus, due to the limitation of memory availability, the length of the model has to be much less than (500 times smaller) the physical length of the channel employed in the experiments. However, since it is experimentally shown that the displacement time difference is not dependant on the length of the capillary or the voltage applied, the numerical model is expected to provide a reasonable representation, at least qualitatively and for the trends of the experimental flow behavior.

7.7.1 Governing Equation

Electric field is applied across the two reservoirs to generate electroosmotic flow and current across the channel. Charge conservation requires that the divergence of current density $\mathbf{i}$ is equal to zero assuming the absence current source/sink in the medium.

$$\nabla \cdot \mathbf{i} = 0 \quad (7.6)$$

In an aqueous solution, the charge carriers are the solute ions. Therefore, in a binary electrolyte system, the current density $\mathbf{i}$ can be related to the transport of ions as follows:

$$\nabla \cdot [\sigma \nabla \phi + F(z_+ D_+ \nabla c_+ + z_- D_- \nabla c_-) - \mathbf{u} F(z_+ c_+ + z_- c_-)] = 0 \quad (7.7)$$

where solution conductivity $\sigma = F(c_+ u_{m+} - c_- u_{m-})$. The current density consists of three components: electromigrative current, diffusive current and convective current. The electromigration of ion is the major contributor to the current observed in the current monitoring experiments and it is often called the conduction current (first term of Eq. 7.7). The diffusive (second term) and convective currents (third term) are neglected because their magnitudes are very small in this case (see Appendix E). Therefore, Eq. 7.7 can be reduced to the Laplace equation (Eq. 7.8) which governs the applied electric potential distribution ϕ .

$$\nabla \cdot (\sigma \nabla \phi) = 0 \quad (7.8)$$

Since the conductivity of the simulation domain is varying throughout the displacement flow, the solution conductivity σ is not a constant but varies with the concentrations of the ions.

The static wall potential distribution ψ is given by the Poisson equation (Eq. 7.9).

$$\nabla \cdot \nabla \psi = \frac{\rho_e}{\epsilon_r \epsilon_o} \quad (7.9)$$

where the net charge density $\rho_e = F(c_+ - c_-)$. It is important to highlight here that the electrical potential is not forced to obey Poisson-Boltzmann equation which is only valid at the steady state condition for a single fluid electroosmotic flow. In two fluid displacement flow, the concentration of the solutions in the channel is not constant, and thus the zeta potential of the channel wall is constantly varying and should not be prescribed *a priori*. Therefore, the wall potential must be allowed to vary according to the local net charge density which is governed by the positive and the negative ions.

The distributions of positive and negative ions (c_+ and c_-) in Eq. 7.9 are not specified but are to be solved by the Nernst-Planck equation (Eq. 7.10 and 7.11).

$$\frac{\partial c_+}{\partial t} + \nabla \cdot [-D_+ \nabla c_+ - u_{m+} c_+ \nabla (\phi + \psi)] = -\mathbf{u} \cdot \nabla c_+ \quad (7.10)$$

$$\frac{\partial c_-}{\partial t} + \nabla \cdot [-D_- \nabla c_- - u_{m-} c_- \nabla(\phi + \psi)] = -\mathbf{u} \cdot \nabla c_- \quad (7.11)$$

Eq. 7.10 and 7.11 show that the ion distribution changes are governed by three components, namely diffusive, electromigrative and convective components. The electromigrative terms consist of two parts: the component due to applied electric field and the component due to the static wall potential, which are summed based on the principle of superposition of electrical potential.

Eq. 7.12 and 7.13 are respectively the Navier-Stokes equation for incompressible Newtonian fluid and the continuity equation which govern the flow field $\mathbf{u}$ and pressure p .

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla) \mathbf{u} = -\nabla p + \mu \nabla^2 + \rho_e [-\nabla(\phi + \psi)] \quad (7.12)$$

$$\nabla \cdot \mathbf{u} = 0 \quad (7.13)$$

The last term of Eq. 7.12 is the body force caused by the applied potential and wall potential on the fluid near the wall with net charge density ρ_e . For microfluidics, the inertial term can be neglected without significant loss of accuracy since Reynolds number is less than 1 (Stokes flow). The values of the various parameters employed are listed in Table 7.2.

Table 7.2: Symbols and values of parameters in numerical models.

Parameter	Symbol (unit)	Value
Faraday constant	F / Cmol^{-1}	96485
Relative permittivity	ϵ_r	80
Permittivity of free space	$\epsilon_0 / \text{Fm}^{-1}$	8.85×10^{-12}
Bulk concentration	c_o / molm^{-1}	0.01
Diffusion coefficient of K^+ ion	$D_+ / \text{m}^2 \text{s}^{-1}$	1.957×10^{-9}
Diffusion coefficient of Cl^- ion	$D_- / \text{m}^2 \text{s}^{-1}$	2.032×10^{-9}
Ion mobility of K^+	$u_{m+} / \text{m}^2 \text{V}^{-1} \text{s}^{-1}$	7.63×10^{-8}
Ion mobility of Cl^-	$u_{m-} / \text{m}^2 \text{V}^{-1} \text{s}^{-1}$	-7.92×10^{-8}
Ion charge number of K^+ ion	z_+	+1
Ion charge number of Cl^- ion	z_-	-1
Temperature	T / K	298
Electron charge	e / C	1.602×10^{-19}
Boltzmann constant	$k_b / \text{m}^2 \text{kg s}^{-2} \text{K}^{-1}$	1.381×10^{-23}
Density of water	ρ / kgm^{-3}	1000
Viscosity of water	$\mu / \text{kgm}^{-1} \text{s}^{-1}$	8.90×10^{-4}
Avogadro constant	N_A / mol^{-1}	6.022×10^{23}

7.7.2 Boundary and Initial Conditions

Boundary conditions for the simulation with displacement flow of a single fluid with concentration 0.01molm^{-3} (0.01mM) are summarized in Table 7.3. The solution concentration in this numerical study is two order of magnitude smaller than the experimental solution concentration. This is because concentration as high as 1mM will require very fine mesh at the vicinity of the shear plane to resolve the steep change in ion concentration, pressure and velocity due to the extremely thin EDL layer. This would require excessive amount of computation time and memory that our current computational resources will not allow. However, we note that the characteristic length scale of EDL is given by the Debye length

$$\lambda_D = \left(\frac{\epsilon_r \epsilon_0 k_b T}{2 z^2 e^2 N_A c_o} \right)^{1/2} \quad (7.14)$$

λ_D for a 0.01mM solution is calculated to be 97.2nm which is still much smaller than the radius of the simulation domain (10 μ m). Therefore, no overlapping of EDL is expected and the simulation should give a good representation of the flow of higher concentration solution in a micro-channel. Thus, for this preliminary study to provide an understanding of the mechanism of the two fluid displacement flow behavior, there will be no loss of generality by setting the solution concentration for the simulation at 0.01 mM.

Table 7.3: Summary of boundary conditions for steady state numerical model for single fluid electroosmotic flow

Variable	Condition	Boundary
Applied potential ϕ	$\phi = 1V$	Inlet
	$\phi = 0$	Outlet
	$\mathbf{n} \cdot \nabla \phi = 0$	Wall, axis of symmetry
Electrostatic potential ψ	$\mathbf{n} \cdot \nabla \psi = 0$	Inlet, outlet, axis of symmetry
	$\mathbf{n} \cdot \nabla \psi = -\frac{0.7 \times 10^{-3}}{\epsilon_r \epsilon_0}$	Wall
Positive ion concentration c_+	$c_+ = c_0 \exp\left(-\frac{e\psi}{k_b T}\right)$	Inlet, outlet
	$\mathbf{n} \cdot (-D_+ \nabla c_+ - u_{m+} c_+ \nabla(\phi + \psi) + \mathbf{u} c_+) = 0$	Wall, axis of symmetry
Negative ion concentration c_-	$c_- = c_0 \exp\left(\frac{e\psi}{k_b T}\right)$	Inlet, outlet
	$\mathbf{n} \cdot (-D_- \nabla c_- - u_{m-} c_- \nabla(\phi + \psi) + \mathbf{u} c_-) = 0$	Wall, axis of symmetry
Flow velocity $\mathbf{u}$ and pressure p	$p = \frac{2c_0 F k_b T}{e} \cdot \left[\cosh\left(\frac{e\psi}{k_b T}\right) - 1 \right]$	Inlet, outlet
	$\mathbf{u} = 0$	Wall
	$\mathbf{n} \cdot \mathbf{u} = 0$	Axis of symmetry

Note: $\mathbf{n}$ =unit vector normal to the boundary

A voltage of 2V is applied across the inlet and outlet of the microchannel. The resultant electric field is 125Vcm^{-1} which is equivalent to the experimental scenario of applying 1000V over an 8cm long microcapillary. Insulation and symmetrical boundary conditions are applied at the channel wall and along the line of symmetry respectively. It is assumed that there is no specific ion absorption on the wall and the wall charge is constant during the course of the two fluid displacement flow process. The surface charge σ_w of glass is assumed to be -0.7 mC/m^2 in a solution with concentration of 0.01 mM (Behrens and Grier 2001).

The inlet and outlet of the microchannel are connected to the reservoirs. Neglecting the entrance and exit effects, the positive and negative ion concentration profiles at the inlet and outlet are assumed to follow the Poisson-Boltzmann distribution. Assuming that there is no viscous stress at the inlet and outlet, the steady state pressure at the inlet and outlet can be derived by solving the NS equation (without the inertial terms):

$$0 = -\frac{\partial p}{\partial r} - \frac{\partial \psi}{\partial r} \rho_e \quad (7.15)$$

Substituting the positive and negative ion concentrations (Boltzmann distribution Eq. 7.2) and integrating Eq. 7.15 once yield,

$$p = \frac{2c_0 F k_b T}{e} \left[\cosh\left(\frac{e\psi}{k_b T}\right) - 1 \right] \quad (7.16)$$

No slip boundary condition is specified at the wall.

The simulation domains are meshed with 21000 quadrilateral elements (70 and 300 edge elements in the radial and axial directions respectively). The size of the elements varies exponentially in the radial direction such that the elements near the wall are much finer to capture the steep changes of variables in the EDL layers. The electrostatic potential, ion concentration, and applied potential are discretized with second order elements while the pressure and velocity are discretized with linear elements. Convergence test has been performed with higher number of

elements for steady-state solutions (see Appendix D.2).

The numerical solving process is summarized in Figure 7.8. First, the wall static potential distribution and ion distribution are solved with Poisson equation and NP equation, by assuming no fluid flow and no applied electric field. After obtaining the ion distribution, Laplace equation can then be solved to obtain the applied electric potential distribution. Finally, the continuity and NS equations are solved to obtain the flow field and pressure in the channel. Convective component due to fluid flow and electromigrative component due to applied electric field are then added to the NP equation. All these equations are then solved simultaneously to obtain the steady state solution of a single fluid electroosmotic flow. The solving and iteration processes are handled by the stationary solver of COMSOL and convergence is obtained within 25 iterations.

After obtaining the steady state solutions for a single fluid flow, boundary conditions at the inlet are modified to model the time-dependant flow condition of the two fluid displacement flow. The steady state solution is set as the initial condition. The inlet boundary condition for c_+ , c_- and p in a displacement flow with 0.002mM solution displacing 0.01mM solution (80% concentration difference, concentration ratio $R = 5$) are modified according to Table 7.4. However, setting this boundary condition will induce inconsistency with the boundary condition of the steady state solution. To resolve this inconsistency, the value of ion concentration is ramped down from the steady state value $c_0 = 0.01\text{mM}$ to $c_0/5 = 0.002\text{mM}$ in an arbitrarily short time (0.0001s). The time-dependant solution for the flow of 0.002mM solution (inlet reservoir) displacing 0.01mM solution (outlet reservoir) can then be obtained.

Figure 7.8: Flowchart of numerical solving process.

The simulation of displacement flow in the other direction can be performed similarly by first obtaining the steady state solution of a single fluid flow for 0.002 mM solution. Subsequently, the inlet boundary condition is similarly modified by ramping up from the steady state value $c_0/5 = 0.002 \text{ mM}$ to $c_0 = 0.01 \text{ mM}$ in 0.0001s. Displacement flows with other pair of solutions (0.005 molm^{-3} or 0.0095 mM with 0.01mM) are performed in a similar manner.

Table 7.4: Changes in inlet boundary conditions for two-fluid displacement flow (R_c = concentration ratio between two solutions)

Variable	Inlet boundary condition
Positive ion concentration c_+	$c_+ = \frac{c_0}{R_c} \exp\left(-\frac{e\psi}{k_b T}\right)$
Negative ion concentration c_-	$c_- = \frac{c_0}{R_c} \exp\left(\frac{e\psi}{k_b T}\right)$
Flow velocity $\mathbf{u}$ and pressure p	$p = \frac{2c_0 F k_b T}{e R_c} \left[\cosh\left(\frac{e\psi}{k_b T}\right) - 1 \right]$

7.8 Numerical Results

The snapshots for the simulated spatial-temporal concentration distribution during the displacement process are shown in Figure 7.9. The ion concentration distributions along the middle of the channel (bulk) at various times are shown in Figure 7.10. It is noted that the initial concentration distribution along the middle of the channel (bulk) resembles a tanh or exponential curve. However, the profile changes significantly as the displacement process occurs. Eventually the shape of the interface is distorted by the induced pressure gradient and axial/radial diffusion across the interface. Therefore, the fluid interface during the displacement flow of two fluids with a concentration mismatch is neither sharp nor straight. The equivalent displacement of the fluid interface X is defined as

$$X = \int u_{av} dt \quad (7.17)$$

where the average x-velocity $u_{av} = Q/A_c$, and A_c =cross section area of the channel. The flow rate Q for the displacement flow of 0.01mM and 0.002mM (80% concentration difference) is obtained by

integrating the x-velocity over the cross section area of the channel. The flow rates for the displacement flow of these two directions are time-dependant and bounded by the two single fluid flow rates (see Figure 7.11a). Therefore X can be obtained by integrating the flow rate Q with respect to time (or area under the curve of Figure 7.11a) and dividing it by A_c .

Figure 7.9: Snapshots of numerically simulated negative ion distribution during displacement flow process of 0.01mM and 0.002mM KCl solutions. Flow direction is from left to right for both cases. Color scale on right represents concentration of negative ion from 0 to 0.01mM.

Figure 7.10: Simulated negative ion concentration along centre line of microchannel during displacement flow process of (a) 0.01mM KCl displacing 0.002mM KCl (b) 0.002mM KCl displacing 0.01 mM KCl at various times. Flow direction is from left to right in both cases.

The displacement time can be obtained by determining the time required for the fluid interface to travel 1.6×10^{-4} m which is the length of the microchannel (see Figure 7.11b). The single fluid flow displacement times for 0.01mM and 0.002mM solutions are 0.2345s and 0.156s respectively. The displacement increases linearly with time for a single fluid flow. In contrast, the displacement-time relationship for two fluid displacement flow is found to be non-linear (see Figure 7.11b). The displacement time for the case of 0.002mM displacing 0.01mM (T_{LH}) and the case of 0.01mM displacing 0.002mM (T_{HL}) are 0.194s and 0.1745s respectively. The simulation result shows that $T_{LH} > T_{HL}$ and this trend agrees with the experimental findings in Section 7.6. The percentage time difference between the flows of these two directions is 11.2%.

Figure 7.11: Numerical results showing (a) flow rate and (b) displacement of fluid interface in displacement flow of 0.01mM and 0.002mM solutions. Single fluid flows for each of these two solutions are shown for reference.

Figure 7.12 shows a comparison between current-time curve obtained from experimental and simulation results. As the length of channel and applied voltage are different in both cases, comparison is achieved through normalization of parameters. The experimental and numerical currents are normalized with the maximum and minimum currents in the experiment and simulation respectively such that all values of current fall between 0 and 1. The numerical

displacement times for both cases of 0.002mM displacing 0.01mM and 0.01mM displacing 0.002mM are normalized with the time to reach the steady current for the former case. The experimental displacement times for the displacement flow of 0.2mM and 1mM are normalized in a similar manner. The numerical results illustrate that, the time for a high concentration solution displacing low concentration solution is indeed shorter than the displacement time for the flow in the reverse direction. This trend agrees with the experimental results. However, as expected, quantitative agreement is not exactly good as expected as the numerical solution concentrations in both cases differ by two order of magnitude with the experimental solution concentrations .

Figure 7.12: Comparison between experimental and numerical results for displacement flow of two solutions with 80% concentration difference. Currents are normalized with maximum and minimum currents. Time is normalized with time for descending curve to reach a steady current.

Despite of the lack of good quantitative agreement between experimental results and simulations, these simulations do reveal the mechanism for the time difference in two fluid displacement flow with large concentration difference. Figure 7.13 shows positive and negative ion concentration distributions at $x=0.8 \times 10^{-4}$ m (half of the channel length) during the two-fluid displacement flow at various times corresponding to various specific bulk concentrations at the line of symmetry evolution. More specifically, these curves are snapshots of the radial concentration profiles when the bulk concentrations (concentrations at the line of symmetry) are 0.002mM, 0.004mM, 0.006mM, 0.008mM and 0.01mM.

Figure 7.13: Positive and negative ion distributions $x=0.8 \times 10^{-4}$ m for flow of (a) 0.002mM displacing 0.01mM and (b) 0.01mM displacing 0.002mM. Full curves represent negative ion and dashed curves represent positive ion.

The steady state concentration profiles for a single fluid flow is illustrated in Figure 7.13 as curve $t=0$. Positive ion concentration decreases and negative ion concentration increases with increasing distance from the wall. The concentrations of positive and negative ions coincide at the EDL edge where the net charge becomes zero. Beyond the EDL, the steady state curves are flat and

there is no concentration variation. The plug-like ionic concentration and velocity profile agree with the conventional electroosmotic models. The solution with a lower concentration produces a thicker EDL and results in a higher flow rate.

The concentration and velocity profiles for two fluid displacement flow deviate significantly from the typical plug-like profile of a single fluid electroosmotic flow. Solutions with different concentration flow at different velocities. To maintain continuity, a pressure gradient is generated and the velocity profile is no longer plug-like (see Figure 7.14). The velocity profile is a superposition of the plug-like profile of electroosmotic flow and the parabolic profile due to pressure driven flow. This shows that the numerical simulation generate the bulk flow profiles which agree with the conventional theory in the literature (Herr *et al.* 2000; Minerick *et al.* 2002). However, hitherto there is no study on the effect of the flow profile distortion, the induced convection and diffusion effects on the ion distribution in EDL layer.

The variation of velocity and the diffusion induced by the concentration gradient at the interface of the two fluids affect the ion distributions due to the presence of the convective and diffusive components in the Nernst-Planck equation. Positive and negative ion concentrations are shown to vary with distance even beyond the edge of EDL (see Figure 7.13) while maintaining zero net charge (positive ion and negative ion curves overlap).

The ion concentration profiles for the flows in both directions are plotted together for ease of comparison (only negative ion is shown). The concentration at the edge of EDL is higher than the bulk concentration for the case of 0.002mM displacing 0.01mM whereas the concentration at the edge of EDL is lower than the bulk concentration for the case of 0.01mM displacing 0.002mM. A lower ion concentration near the wall results in a thicker EDL and hence higher flow rate. The reverse is true if the ion concentration is higher near the wall. It is this different distortion of ionic concentration profile at the EDL that induces the displacement time difference.

Figure 7.14: Snapshots of velocity vector plot for flow of (a) 0.002mM displacing 0.01mM and (b) 0.01mM displacing 0.002mM. Numerical results show that velocity profiles in two fluid displacement flow deviate from plug-like profile of typical electroosmotic flow.

Figure 7.15: Comparison of negative ion distributions between two flow directions at bulk concentration of 0.004mM, 0.006mM and 0.008mM at $x=0.8 \times 10^{-4}$ m. Full curves represent the case of high concentration displacing low concentration and dashed curves represent the case of low concentration displacing high concentration.

Simulations for the displacement flow of 0.0095mM and 0.005mM solutions with 0.01mM solution (5% and 50% concentration differences) have also been performed. Percentage time difference for the displacement flow of solutions with 50% concentration difference is calculated to be 2.9% while the time difference for the case of 5% concentration difference is negligible (less than 1%). This shows that the displacement time difference diminishes with decreasing concentration difference. This trend is logical and agrees with the experimental results. However, it should be noted that the exact percentage time differences obtained from experiments and simulations are not expected to be the same because the concentrations of solutions between simulations and experiments are significantly different.

7.9 Summary

Electroosmotic displacement flow involving two dissimilar fluids is commonly encountered in microfluidics. The flow direction dependence of displacement time was investigated experimentally. The displacement time was determined with current monitoring method. It was established experimentally that the time required for a high concentration solution to displace a low concentration solution is less than the time required for a low concentration solution to displace a high concentration solution. It was found that the percentage displacement time difference increases with increasing concentration difference. If the concentration difference between the two solution is large (>30% for glass channel and >50% for PDMS channel), the displacement time difference between these two directions is not negligible. The percentage time difference could be as high as 28.4% if the concentration ratio between the two solutions is 5:1. The percentage time difference appears to be constant regardless of the length or diameter of the channel and the voltage applied.

If the concentration difference between the two fluids is small (less than 5%), the displacement time difference in both directions are negligible. Current monitoring technique to measure zeta potential typically involves the displacement flow of two solutions with approximately 5% concentration difference. Therefore, the accuracy of measured zeta potential value based on the displacement time in any directions is acceptable and well within experimental error.

A numerical model has been proposed to investigate and simulate this phenomenon. Simulation results show that the velocity profile and ion distribution differ significantly from those of single fluid electroosmotic flow. The distortion of ionic profiles near the EDL is responsible for the displacement time difference for the two different flow directions. The percentage time difference increases with increasing concentration difference. The trends obtained from simulations agree qualitatively with experimental observations. These simulations provide the necessary explanations and the mechanism for the experimental findings.

Chapter 8 Conclusions and Future Studies

8.1 Research Contributions

This thesis presented a novel micromixing scheme with a simple yet efficient mechanism to enhance mixing efficiency in microfluidic channels. The concept is based on and demonstrated in a T-channel with a constriction introduced at the T-junction. Two out-of-phase sinusoidal electric fields drive the fluids through the channel and simultaneously induce oscillation of the fluid interface at the T-junction. These oscillations create alternate plugs of fluids in the constriction. These plugs of fluids spread into thin crescent shaped layers with a substantial increase in contact surface areas between the two fluids and a considerable reduction in mixing length, resulting in significant enhanced mixing.

One of the merits of the proposed micromixer is its two-dimensional design, which simplifies the fabrication procedures and facilitates mass production process. Furthermore, since no embedded electrode and metallic structure are required, it can be integrated into various microfluidic devices easily and cheaply. The amplitude and frequency of the applied AC voltage can be fine-tuned to maximize the mixing efficiency under various flow rates. The micromixer does not demand a high applied voltage or impose any restriction on the properties of the solutions.

Various characteristic parameters that govern the mixing efficiency, such as applied AC amplitude and frequency, location and length of constriction were identified analytical and investigated experimentally. The understanding of the effect of these parameters on the mixing enhancement

mechanism is crucial in optimizing the mixing performance of the proposed micromixer. Numerical studies were performed to obtain an accurate representation of the flow condition in the micromixer.

Despite the abundance of charged (ionic) solutes which are commonly mixed in microfluidic devices, most researchers employ a neutral dye in their experimental investigations or neglect the ionic mobility of dye/solute in their numerical studies. The mobility of fluorescent dye or any ionic solute to be mixed is found to be important in achieving accurate simulation of the mixing condition under electroosmotic flow. It is concluded that, under electrokinetically driven flow, the concentration distribution and mixing of ionic or charged solutes are strongly dependent on the mobility of the solutes. Failure to account for the ionic mobility will result in significant error in the simulations which could deviate considerably from experimental flow behavior.

In view of the importance of the solute mobility in mixing simulation, a simple method adapted from the micromixing scheme has been proposed to measure the mobility of the fluorescent dye or solute. The mobilities of the fluorescent dye in various buffer concentrations have been measured and their values agree well with the experimental and theoretical values found in the literature. This method provides an alternative to the conventional method of measuring ionic mobility of fluorescent ion species. This method of mobility measurement can potentially be employed to measure the mobility of non-fluorescent ion species with different sensors.

Flow simulation in an electrokinetically driven micromixer often requires the zeta potential of the microchannel to be specified as the boundary condition. One of the common methods to measure zeta potential is through current monitoring method. The current monitoring experiment involves the displacement flow of two fluids with different concentrations. An anomaly whereby the displacement time is dependent on the flow direction has been observed. Hitherto, no theoretical or numerical studies have been conducted to understand the mechanism behind this phenomenon. This has

significant implication as the difference in displacement time will result in different zeta potential measured by the current monitoring technique. It is concluded that the displacement time difference is not negligible when the concentration difference between the two solutions are large (>5%). A numerical model has been implemented to describe and explain the phenomenon. The displacement time difference is caused by the distortion of ion concentration due to diffusive and convective effects near the electrical double layer.

8.2 Recommendations for Future Studies

Some recommendations for future studies include:

- 1) Micromixing under sinusoidal pressure driven flow – The micromixing technique proposed in this thesis has been demonstrated with electroosmotic flow. However, the flow rate for electroosmotic flow is limited because high voltage would induce severe Joule heating and bubble creation would disrupt the fluid flow. Instead of applying electric fields, it would be beneficial to apply sinusoidal pressures (with peristaltic pump for example) to investigate the mixing condition for a range of flow rate, from low (< 1) to high (> 40) Reynolds number. The transition from stable to unstable flow with various parameters such as frequency and amplitude of oscillation for these flow rates can be investigated.
- 2) Mobility measurement – The operating parameters for the mobility measurement technique proposed in this thesis should be optimized to generate shaper signal for more accurate measurements. The possibility of extending this method to measure mobility of non-fluorescent species with different sensors can be explored. The measurement of the fluid interface oscillation amplitude can also be a potential method to measure ionic mobility if electromigrative effect is included in the analysis in Chapter 3.

- 3) Two fluid displacement flow simulation for high concentration solutions – Two fluid displacement flow simulation was performed with only low concentration solutions due to the limitation in computational resources. This simulation could be extended to high concentration solutions if the appropriate computation resources, both in terms of computational speed and memory, are available.
- 4) Velocity measurement in EDL in two fluid displacement flow – The zeta potential and velocity in the EDL layer is changing during the two fluid displacement flow. It would be interesting to measure the changes of velocity with surface observation technique such as total internal reflection fluorescent microscopy (TIRFM) which is based on evanescent waves. Other possible mechanisms which may induce the hysteresis effect such as charge development at the interface between two fluids with different conductivity (Ren *et al.* 2009) will also be explored.

List of Publications

Journal Paper

1. Lim, C. Y., Y. C. Lam and C. Yang (2010). "Mixing enhancement in microfluidic channel with a constriction under periodic electro-osmotic flow." Biomicrofluidics **4**(1): 014101.
2. Lim, C.Y. and Y.C. Lam (2012). "Analysis on micro-mixing enhancement through a constriction under time periodic electroosmotic flow." Microfluidics and Nanofluidics **12**(1): 127-141.
3. Lim,C.Y. and Y.C. Lam (2012). "Direction dependence of displacement time for two-fluid electroosmotic flow." Biomicrofluidics **6**(1): 012816.

Conference Paper

1. Lim,C.Y. and Y.C. Lam (2011). "Cross-shaped Micromixer with Constriction under Periodic Electroosmotic Flow." 2nd Conference on Advances in Microfluidics and Nanofluidics and Asian-Pacific International Symposium on Lab on Chip.

[Oral presentation, 5th Jan – 7th Jan 2011, Singapore]

Appendix A: Fluid Interface Fluctuation

In a simple T-mixer, the applied voltages at the two side channels drive fluids from the inlet reservoirs towards the main channel (see Figure A.1). The two fluid streams from the two side channels converge at the T-junctions and mix. If only DC electric field is applied, the position of the confluent fluid interface remains constant along the y-axis, assuming both streams have identical flow velocity.

Figure A.1: Coordinate system at the T junction

If the applied voltages are time periodic and are not in-phase, the position of the fluid interface will not be stationary. The movement of the fluid interface is caused by the varying strength of the electric field applied at the two side channels. For this investigation, the applied electric fields are assumed to be sinusoidal with angular frequency ω , AC amplitude E_{AC} and DC offset (bias) E_{DC} . The electric fields applied at the two side channels are assumed to be out-of-phase by 180° . An expression

describing the position of the confluent fluid interface at the T-junction can be derived. In this analysis, only the x component of the electric field is considered. The instantaneous electric field at the T-junction, $E(t)$ can be approximated as a sum of the DC electric field and the AC electric field.

$$E(t) = E_{AC} \sin(\omega t) - E_{AC} \sin(\omega t - \pi) - Gx$$

$$E(t) = 2E_{AC} \sin(\omega t) - Gx \quad (\text{A.1})$$

The first term in Eq. A.1 is the difference of the two AC electric fields which are out of phase by 180° while the second term is the DC electric field. The DC electric field is approximated as a linear function of position, x , with D as the constant of proportionality. The DC electric field is in fact not uniform at the T-junction. This approximation can be regarded as the first term of the Taylor series expansion for the electric field.

With Helmholtz-Smoluchowski equation, the governing equation for the position of the interface of the confluent streams can be written as,

$$\frac{dx}{dt} = \frac{-\varepsilon_r \varepsilon_0 \zeta}{\mu} [2E_{AC} \sin(\omega t) - Gx]$$

$$\frac{dx}{dt} - \frac{\varepsilon_r \varepsilon_0 \zeta G}{\mu} x = \frac{-2\varepsilon_r \varepsilon_0 \zeta}{\mu} E_{AC} \sin(\omega t) \quad (\text{A.2})$$

Let $p = -\frac{\varepsilon_r \varepsilon_0 \zeta G}{\mu}$ and $q = \frac{-2\varepsilon_r \varepsilon_0 \zeta}{\mu} E_{AC}$, Eq A.2 simplifies to

$$\frac{dx}{dt} + px = q \sin(\omega t) \quad (\text{A.3})$$

Multiplying Eq. A.3 by e^{pt} gives,

$$e^{pt} \frac{dx}{dt} + e^{pt} px = e^{pt} q \sin(\omega t)$$

$$\frac{d}{dt}(e^{pt}x) = qe^{pt} \sin(\omega t) \quad (\text{A.4})$$

Integrating Eq. A.4 with respect to t yields

$$e^{pt}x = \int qe^{pt} \sin(\omega t) dt \quad (\text{A.5})$$

Performing integration by parts,

$$e^{pt}x = \frac{qe^{pt}}{p} \sin(\omega t) - \frac{q\omega}{p} \int e^{pt} \cos(\omega t) dt + c_1 \quad (\text{A.6})$$

where c_1 is the integration constant. Integrate the second term on the right hand side of Eq. A.6 by parts again, gives

$$\int qe^{pt} \sin(\omega t) dt = \frac{qe^{pt}}{p} \sin(\omega t) - \frac{q\omega e^{pt}}{p^2} \cos(\omega t) - \frac{q\omega^2}{p^2} \int e^{pt} \sin(\omega t) dt + c_2 \quad (\text{A.7})$$

where c_2 is the combined integration constant after the two integrations. Note that the second last term on the right side of Eq. A.7 is in a form similar to the original integration. Rearranging the equation yields,

$$\int qe^{pt} \sin(\omega t) dt = \frac{p^2}{p^2 + \omega^2} \left[\frac{qe^{pt}}{p} \sin(\omega t) - \frac{q\omega e^{pt}}{p^2} \cos(\omega t) + c_2 \right] \quad (\text{A.8})$$

Therefore, the original integration can be evaluated as,

$$e^{pt}x = \int qe^{pt} \sin(\omega t) dt$$

$$e^{pt}x = \frac{p^2}{p^2 + \omega^2} \left[\frac{qe^{pt}}{p} \sin(\omega t) - \frac{q\omega e^{pt}}{p^2} \cos(\omega t) + c_2 \right] \quad (\text{A.9})$$

Dropping e^{pt} from all the terms of Eq. A.9 gives,

$$x = \frac{p^2}{p^2 + \omega^2} \left[\frac{q}{p} \sin(\omega t) - \frac{q\omega}{p^2} \cos(\omega t) + c_2 e^{-pt} \right] \quad (\text{A.10})$$

With initial conditions of $t=0$, $x=0$, the integration constant c_2 can be evaluated ,

$$c_2 = \frac{q\omega}{p^2} \quad (\text{A.11})$$

x is then given by,

$$x(t) = \frac{pq}{p^2 + \omega^2} \sin(\omega t) - \frac{q\omega}{p^2 + \omega^2} \cos(\omega t) + \frac{q\omega e^{-pt}}{p^2 + \omega^2} \quad (\text{A.12})$$

It should be highlighted here that the last term on the right side of Eq. A.12 quickly diminishes as t increases. As such, at steady oscillation ($t \rightarrow \infty$), the equation can be written as,

$$x(t) = \frac{pq}{p^2 + \omega^2} \sin(\omega t) - \frac{q\omega}{p^2 + \omega^2} \cos(\omega t) \quad (\text{A.13})$$

Eq. A.13 can be written in the form of $x(t) = A \sin(\omega t - \phi)$ for easier interpretation. A can be regarded as the amplitude of fluctuation for the interface of the confluent streams. Let

$$A \cos \phi = \frac{pq}{p^2 + \omega^2} , \quad A \sin \phi = \frac{q\omega}{p^2 + \omega^2} \quad (\text{A.14})$$

With the trigonometry identity based on Eq. A.14, A can be expressed as,

$$\therefore A^2 (\sin^2 \phi + \cos^2 \phi) = \left(\frac{pq}{p^2 + \omega^2} \right)^2 + \left(\frac{q\omega}{p^2 + \omega^2} \right)^2$$

$$A = \frac{q}{\sqrt{p^2 + \omega^2}} \quad (\text{A.15})$$

Similarly from Eq. A.14, ϕ can be expressed as,

$$\tan \phi = \frac{\omega}{p} \quad (\text{A.16})$$

Hence, Eq. A.13 can be rewritten as,

$$x(t) = \frac{q}{\sqrt{p^2 + \omega^2}} \sin \left[\omega t - \tan^{-1} \left(\frac{\omega}{p} \right) \right] \quad (\text{A.17})$$

Substituting p and q into Eq. A.17 gives,

$$x(t) = \frac{-2\varepsilon_r \varepsilon_0 \zeta E_{AC} / \mu}{\sqrt{(-\varepsilon_r \varepsilon_0 \zeta G / \mu)^2 + \omega^2}} \sin \left[\omega t - \tan^{-1} \left(\frac{-\mu}{\varepsilon_r \varepsilon_0 \zeta G} \right) \right] \quad (\text{A.18})$$

Appendix B: Stroboscopic Effect for Capturing of Periodic Flow Dynamics

In Section 4.5, a CCD camera with 5 fps was able to capture flow dynamics such as fluid interface oscillation under various frequencies based on stroboscopic effect. A brief discussion on the stroboscopic effect for the capturing of the dynamics of *periodic* flow behavior is explained here.

If the sampling frequency, f_{sampling} is set to the same frequency as the observation/signal frequency, f_{signal} (for the micromixing experiment, the observation/signal would be the mixing images or the fluid interface fluctuation), the captured image will be a stationary one. Figure B.1 shows an example for the case of $f_{\text{signal}} = 5\text{Hz}$ and $f_{\text{sampling}} = 5\text{Hz}$, which is the capturing frequency of the CCD camera.

Figure B.1: Sampling frequency $f_{\text{sampling}} = \text{Signal frequency } f_{\text{signal}} = 5\text{Hz}$. Signal points are always captured at the same position/phase of a cycle.

If the observed signal oscillates at other frequency, for example, $f_{signal}=4\text{Hz}$, and $f_{sampling} = 5\text{Hz}$, the captured image will repeat itself after every 5 images (see Figure B.2). This is because the period for the signal ($1/f_{sig} = 1/4$) and the sampling period ($1/f_{sampling} = 1/5$) have a least common multiple (LCM) of 1. The number of images required for the captured images to repeat itself, n is given by

$$n = \text{LCM} * f_{sampling} \quad (\text{B.1})$$

In fact, if $f_{sampling}= 5\text{Hz}$, n will be 5 as long as f_{signal} is an integer (except for 5 or its multiples) since their LCM with the sampling period are also 1. This is undesirable because the images captured do not show the full dynamics of the fluid oscillation interface or mixing condition.

The CCD camera employed in the experiment was only able to capture image up to 5fps and thus $f_{sampling}$ was restricted to only 5Hz. However, the input signal can be modified. In the experiments, the voltage frequency f_{signal} was set to 2.1Hz, 4.1Hz, 6.1Hz, 8.1Hz, 12.1Hz or 16.1Hz. The LCM's for these capturing periods and the signal period are calculated to be 10. Thus, it will take $10 \times 5 = 50$ images before these images repeating themselves. Therefore, each of these 50 images shows the mixing image and fluid interface position at a *different* phase in a cycle (see Figure B.3). Therefore, the dynamics of the fluid interface fluctuation and mixing condition at different phases in a cycle can be captured and analyzed, even with a slow capturing frequency of just 5Hz.

Figure B.2: Sampling frequency $f_{\text{sampling}} = 5\text{Hz}$. Signal frequency $f_{\text{signal}} = 4\text{Hz}$. The captured signal points repeat themselves after every 5 sampling. The captured signal points at the same phase/position are marked with the same alphabets (A,B,C,D,E) for clarity.

Figure B.3: Sampling frequency $f_{\text{sampling}} = 5\text{Hz}$. Signal frequency $f_{\text{signal}} = 4.1\text{Hz}$. The captured signal points appear at different phases/positions in a cycle and will only repeat themselves after 50 samplings.

Appendix C: MATLAB Script

The mixing efficiencies of the micromixer employed in the experimental investigation in Chapter 5 were evaluated with a Matlab program. An example of the Matlab script for mixing evaluation is shown below.

```
clear all;
close all;
clc;

%~~~ Assigning filename of images

datanum=250;
a = 'file00';
a1= 'file0';
a0='file000';
for i=1:datanum
    b = num2str(i);
    if i < 100
        temp= strcat(a,b);
    else
        temp=strcat(a1,b);
    end
    if i<10
        temp=strcat(a0,b);
    end
    file(i) = {temp};
end
```

```

startpic =1;
endpic = 50;

%~~~ Show first image

A = imread(char(file(startpic)),'BMP');

cmax=max(max(A));
cmin=min(min(A));

image_handle=figure;
A=wiener2(A,D);

imshow(A,[cmin cmax]);

%~~~ Define Max and Min Intensity

cmax =105;
cmin =14;

hold on;

%----Define Mixing Efficiency Evaluation Area

xref = 0;
yref = 380.5;

```

```

pertuabarecdwn = [xref+30,yref,1300,300;];

downrec = rectangle('Position',pertuabarecdwn,'EdgeColor','r');

perturba =[];

ARcnormQ=[];

downloc = [];

hold off;

%~~~ Evaluate Mixing Efficiency

for i = startpic:endpic
    A = imread(char(file(i)),'BMP');
    A = wiener2(A,[5 5]);
    Q = imcrop(A,pertuabarecdwn);
    McminQ = cmin*ones(size(Q,1),size(Q ,2));
    Qnorm= (double(Q) - double(McminQ))/(cmax-cmin);
    ARcnormQ = cat(3,ARcnormQ,Qnorm);
end

clear Qnorm Q A;

numpic = endpic-startpic+1;

Vdevia =ARcnormQ -
0.5*ones(size(ARcnormQ,1),size(ARcnormQ,2),size(ARcnormQ,3));

clear ARcnormQ McminQ;

Vdeviasqr = Vdevia.^2;

Mxsectsqr = mean(Vdeviasqr,3);

```

```

Vxsectmix = ones(1,size(Mxsectsqr,2))-
sqrt(sum(Mxsectsqr,1)/size(Mxsectsqr,1))/0.5;

clear Mxsectsqr;
clear Vdeviasqr;

%~~~ Plot Mixing Efficiency versus Downstream Distance

for i=1:size(Vxsectmix,2)
    downloc(i) = i/300;
end

figure;
plot(downloc,Vxsectmix);
Vxsectmix=Vxsectmix';

```

Appendix D: Convergence Test for FEM Simulation

Numerical simulations of flow in microchannel have been performed with finite element method (FEM) in the thesis. A fundamental premise of FEM is that the simulation domain is sub-divided into small discrete regions known as finite elements. Fine meshing ensures that the finite elements are able to capture the changes of the variables of interest accurately throughout the modelling domain. However, the finer the mesh, the higher is the computational load. Convergence test needs to be performed to ensure that the finite elements are fine enough to produce reliable results without incurring excessive computation cost.

D.1 Micromixing Simulation

The convergent test was performed with 4237, 8891, 17394, 29585 and 34437 triangular elements for the steady-state solution. The values of electric potential, velocity and concentration were determined at a point located at the line of symmetry 1.2mm from the T-junction where mixing efficiency was evaluated. The applied potential and velocity did not change significantly with increasing number of elements. Among the three variables, the value of concentration converged at the slowest rate with mesh refinement (see Figure D.1). The value of ion concentration obtained with 17394 elements differed from the values obtained with the finest elements (34437 elements) by only 0.25%. Therefore, the simulation domain was discretized with 17394 elements to maintain good accuracy and to minimize computational load.

Figure D.1: Convergence test for (a) electric potential (b) velocity (c) concentration at coordinate $x = 1.2\text{mm}$, $y = 0$. Range of y-axis is set to $\pm 2\%$ of the value obtained from the finest mesh (34437 triangular elements).

D.2 Two Fluid Displacement Flow Simulation

The convergent test was performed with 8000 (200x40), 15000 (250x60), 21000 (300x70), 30240 (360x84) and 47250 (450x105) quadrilateral elements (numbers in brackets are the number of edge elements along the axial and radial direction respectively) for the steady-state solution. The size of the elements varies exponentially in the radial direction such that the elements near the wall are much finer for capturing the steep changes of variables in the EDL layers. The values of applied electric potential, wall potential, velocity, position and negative ion concentration were determined at a point located $0.2\mu\text{m}$ from the wall (within the EDL region) at half of the channel length ($x=80\mu\text{m}$). The applied potential and negative ion concentration did not change significantly with increasing number of elements. The values of positive and negative ion concentrations, wall potential and velocity gradually converged with mesh refinement (see Figure D.2). The values of wall potential, velocity, positive and negative ion concentrations obtained with 21000 elements differed from the values obtained with 47250 elements (the finest mesh) by 0.3%, 0.48%, 0.002% and 0.003% respectively. Therefore, the simulation domain was discretized with 21000 elements to maintain good accuracy and to minimize computational load.

Figure D.2: Convergence test for (a) positive ion concentration (b) negative ion concentration (c) wall potential (d) applied potential (e) velocity at $r=9.8\mu\text{m}$, $x = 80\mu\text{m}$. Range of y-axis is set to $\pm 2\%$ of the finest mesh (47250 quadrilateral elements).

Appendix E: Diffusive and Convective Current in Electroosmotic Flow

In two-fluid displacement flow under electroosmosis, electric field is applied across the two reservoirs to generate electroosmotic flow and current across the channel. Charge conservation requires that the divergence of current density $\mathbf{i}$ is zero with the absence of current source/sink in the medium.

$$\nabla \cdot \mathbf{i} = 0 \quad (\text{E.1})$$

In an aqueous solution, the charge carriers are the solute ions. Therefore, in a binary electrolyte system, the current density $\mathbf{i}$ can be related to the transport of ions as follows:

$$\nabla \cdot [\sigma \nabla \phi + F(z_+ D_+ \nabla c_+ + z_- D_- \nabla c_-) - \mathbf{u} F(z_+ c_+ + z_- c_-)] = 0 \quad (\text{E.2})$$

where solution conductivity $\sigma = F(c_+ u_{m+} - c_- u_{m-})$. The current density consists of three components: electromigrative current, diffusive current and convective current. The electromigration of ion (the first term on the left hand side of Eq. E.2) is the major contributor to the current observed in the current monitoring experiments and it is often called the conduction current.

A numerical simulation was conducted to estimate the magnitude of the diffusive and convective currents relative to the conduction current in a two-fluid electroosmotic flow. For simplicity, only the axial component (x direction) of the current density will be modeled because the radial component of the current is not detectable. Assuming the solute is KCl with $z_+ = +1$ and $z_- = -1$, Eq. E.2 can be simplified to

$$\nabla \cdot \left[\sigma \frac{d\phi}{dx} + F(D_+ \frac{dc_+}{dx} - D_- \frac{dc_-}{dx}) - uF(c_+ - c_-) \right] = 0 \quad (\text{E.3})$$

The value of other constants can be found in Table 7.2 of Chapter 7. The diffusive current (second term in Eq. E.3) due to concentration gradient along the channel is very small in this case because the diffusion coefficients of K^+ and Cl^- ions are almost similar. The convective current (the third terms on the left side of Eq. E.3) is several orders of magnitude smaller than the conduction current and is often neglected in the current monitoring experiment (Arulanandam and Li 2000) because the concentration of the positive and negative ions in the bulk are the same (no net charge).

Eq. E.3 is strongly coupled to the Poisson-Nernst-Planck equation, Navier-Stokes equation and continuity equations due to the dependence of total current density on solution conductivity, velocity and positive and negative ion concentration gradients. All the mentioned equations need to be solved simultaneously. Due to the heavy computation load, only the case of 0.01mM KCl displacing 0.002mM KCl (largest axial concentration gradient) with diffusive and convective currents was conducted for a comparison with the simulation results without the diffusive and convective currents.

Figure E.1 indicates that except for the slight deviation at the start of the simulation, the simulated total current with and without diffusive and convective currents are almost similar (the two curves overlap each other). Therefore, it can be concluded that, diffusive and convective currents can be neglected without compromising the accuracy of the numerical simulation to enhance the computation efficiency.

Figure E.1: Total current obtained from numerical simulation with and without convection and diffusive current in two fluid displacement flow (0.01mM KCl displacing 0.002mM KCl). Upper and lower dash lines show total current when channel is filled with 0.01mM and 0.002mM KCl solution respectively.

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